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RNA CONTENT IN MOTOR AND SENSORY NEURONS AND SURROUNDING NEUROGLIA OF MOUSE SPINAL CORD UNDER CONDITIONS OF HYPODYNAMIA AND FOLLOWING NORMALIZATION

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RNA CONTENT IN MOTOR AND SENSORY NEURONS AND
SURROUNDING NEUROGLIA OF MOUSE SPINAL CORD
UNDER CONDITIONS OF HYPODYNAMIA AND FOLLOWING
NORMALIZATION

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Male white mice were kept for 2 and 3 weeks in individual
narrow chambers significantly restricting the movements of animals.
The mice were decapitated immediately after a 2 or 3 week hypody-
namia, as well as 2, 6, 24 and 72 hours after a 3-week hypodynamia.

Using a two-wave length variant of Caspersson's ultraviolet cyto-
spectrophotometry the RNA amount per cell has been determined in
the cytoplasm of the neurons and in the body of their glial cells-
satellites of spinal cord anterior horns and spinal ganglia. Each
cell was photometered twice: before and after the selective HClO4-
extraction of RNA according to the authors' method. It has been
found that 2- and 3-week hypodynamia does not influence the RNA
content in the neurons and glia of spinal cord anterior horns as
well as in the spinal ganglia neurons; in the neuroglia of spinal
ganglia an increase of the RNA content has been noted after a
2-week hypodynamia, and a decrease after 3 weeks. On ceasing the
hypodynamia the RNA content initially turned out to be markedly
decreased both in the neurons and in the neuroglia of anterior
horns and spinal ganglia, later on the RNA amount returned to the
normal level with a hypercompensation, in some cases in the form
of a temporary increase above the normal level. The dynamics of
this restoration of the RNA content in the nerve and glial cells
was somewhat different in the spinal cord anterior horns and in
the spinal ganglia. The rate of the normal RNA level restoration
after the cease of hypodynamia was higher in the glial cells than
in the neurons. After 72 h the RNA content in the cytoplasm of
both types of the neurons studied became normal, while in both
types of neuroglia it decreased again though to a lesser degree
than previously, 2 h after the end of hypodynamia. The results obtained have been compared with the authors' earlier data. On the basis of this comparison the authors discuss the differences in the dynamics of reparative processes in RNA metabolism within the neuron-neuroglia unit after the cessation of hyper- and hypodynamia. The role of neuroglia is stressed in compensatory, reparative and trophic processes in the nervous system as well as the possibility in an adaptation at the cellular level.

The vital activity of the animal organism constantly occurs under conditions of greater or lesser motor activity that involves diverse physiological systems. The proprioceptive and interoceptive impulses from these systems to the cells of the nervous system is one of the conditions for the normal functioning of the nervous tissue, and consequently, the normal occurrence of its intracellular metabolism. Therefore the problem of forced hypodynamia has not only a narrow applied or medical, but also a great general biological importance.

Significant restriction of normal mobility for a long period in man and animals results in a number of functional disorders on the part of the nervous system (Graveline et al., 1961; Gerd, 1963; Hatch et al., 1963; Van Reen, 1964; Gurvich and Yefimenko, 1967; Krupina et al., 1967). The question of how deeply these disorders affect the metabolism of the cells in the nervous system has not been studied in detail.

Our experiment of a quantitative cytochemical study of RNA in individual cells of the spinal cord with intensified motor activity of varying origin (Pevzner and Khaydarliu, 1967; Khaydarliu, 1967a; Brumberg, 1968) confirmed the expediency of a comparative study of such objects that differ in function as the motor neurons of the spinal cord anterior horns and the sensory neurons of the spinal ganglia. In the presence of a number of characteristic differences in the functioning, embryogenesis and morphology of these types of neurons they are similar in that respect that both possess a large mass of cytoplasm that dominates over the mass of the cellular nucleus (Khaydarliu, 1967b), are surrounded by glial cell-satellites and are included in the composition of the spinal reflex arch in which

*Numbers in margin indicate pagination in original foreign text.
The neurons of the ganglia play the role of afferent, and the motor neurons of the anterior horns—the role of the efferent structures. Therefore, although anatomically the spinal ganglia are not a part of the spinal cord we considered it possible to use them for comparison with the motor nuclei of the spinal cord, and for the purpose of brevity to conditionally call the neurons of the ganglia the sensory neurons of the spinal cord.

The results of our other studies (Peuzner, 1965, 1967, 1968) as well as the published data (Hyden, 1962, 1964; Hyden and Lange, 1966) are convincing that the metabolism of neurons and neuroglia can be altered in different ways under conditions of oscillation in the intensity of the functional activity of the nervous system. Less attention was paid to the comparative dynamics of the normalization processes in the neural and glial cells. At the same time analysis of this dynamics could yield valuable information about the metabolic features of the neuroglia by promoting further development of the problem on the role of the neuroglia in the functioning of the neuron.

The task of this work was to compare the dynamics of shifts in the RNA content, the most important chemical component of the cell, in the neurons and neuroglia of the spinal cord anterior horns and the spinal ganglia under the influence both of the actual hypodynamia and mainly, the subsequent normalization.

**Technique**

The experiments were conducted on white male mice weighing 28-32 g. Each animal was placed in a separate plexiglass chamber with dimensions 7.0x2.5x2.5 cm, similar to the chambers described by Fedrov and Grishanin (1967). Such chambers sharply restricted the movements of the animals, not resulting, however, in their complete immobilization. The feeding and drinking pattern of the experimental mice was kept the same as in the control animals.

The first several days after placement in the chamber the mice displayed agitation, then gradually became accustomed to the hypodynamic
conditions and further acted calmly, sitting immovably in the chambers. By the end of three weeks of hypodynamia a number of signs of so-called "hypokinetic complex" began to be noticeable in the animals: loss of body weight (by 15-20%), grooves in the rear extremities, disruption in movement coordination. Normal motor activity in these mice was restored only at the end of 2-3 days after cessation of 3 weeks of hypodynamia.

Within 2 and 3 weeks part of the animals were killed, the other part after 3 weeks of stay in the chambers were left under conditions of free motor activity and they were killed in 2, 6, 24 and 72 hours after removal from the chambers. The animals were decapitated without narcosis. The intumescentia lumbalis with the spinal ganglia adjacent to it were fixed according to Brodskiy in a cooled mixture of formalin, ethanol and acetic acid with subsequent sealing in paraffin. In sections 10 µ thick the optic density of RNA in the cytoplasm of neural and the body of glial cells was determined before and after RNA extraction by 16% HClO₄ for 48 hours at 0-4°C (Brumberg and Pevzner, 1966). The measurements of optic density were made with the help of a two-wave variant (Agroskin et al., 1960) of ultraviolet cytospectrophotometry (Caspersson, 1936, 1950) with 265 and 280 µ on a two-wave sensing ultraviolet cytospectrophotometer of Agroskin design; the plan of the instrument and details of the photometry and computations of the RNA concentration have been previously described by Pevzner (1963, 1966). The quantity of RNA in calculation for one cell was found as the product of the RNA concentration per volume of cytoplasm of the neurons or body of the glial cells. The volume was determined according to the formula of the ellipsoid of rotation; the linear dimensions of the cells were measured with the help of a screw-type ocular micrometer MOWL-15X.

Each average amount of RNA content was found according to the data of photometry of 120-150 cells taken from 5-7 animals. All the numerical material was processed statistically according to Student-Fisher.

Results

The RNA content in the spinal cord cells was determined 2-3 weeks after the start of hypodynamia when, judging from the behavior of the animals
they had adapted sufficiently to the living conditions in the close chambers. The results of the studies showed (fig. 1 and 2) that by this time there were no reliable shifts in the content of cytoplasmic RNA either in the motor or in the sensory neurons. In the glial cells adjacent to the outer membrane of the motor neurons in the anterior horns no significant changes were noted in the RNA content either (fig. 1). In the spinal ganglia the glial cell-satellites were characterized by a clear increase in the RNA content in 2 weeks and a decrease—in 3 weeks after the start of hypodynamia (fig. 2).

The removal of the animals from the chambers and the resumption of free motor activity was accompanied by rapid, sharp shifts in the RNA content. As is apparent on figures 3 and 4, already in 2 hours a considerable reduction occurred in the RNA content both in the neural and in the glial cells of the spinal cord anterior horns and the spinal ganglia. Here in both cases the changes in the neuroglia were expressed to a greater degree than in the neurons. Then restoration of the initial RNA level occurred, whereupon in the spinal ganglia and in particular in the spinal cord anterior horns this restoration was completed in the neuroglia earlier than in the neurons. Further the RNA content in both types of cells was characterized in the beginning by an increase in the normal level, and then by a certain decrease; in the neurons of the spinal ganglia the amplitude of these oscillations was lower and the trend towards their damping was expressed more strongly than in the motor neurons of the spinal cord (fig. 3 and 4). In 3 days after cessation of hypodynamia the RNA content both in the motor and in the sensory neurons practically was normalized, in the neuroglia the changes (reduction) in the RNA content were still preserved.
Figure 2. Changes in RNA Content in Neurons and Neuroglia of Sensory Spinal Ganglia in Mice after 2- and 3-Week Hypodynamia
Designations the same as in figure 1.

Discussion

Numerous cytochemical works, among which the systematic studies of Hyden and his colleagues in Sweden are isolated (Hyden, 1962, 1964; Hyden and Lange 1966) and Brodskiy and his colleagues (Brodskiy, 1961, 1966; Brodskiy and Pechayeva, 1958, 1959) have established that excitation of neurons that does not result in their fatigue or exhaustion in the final analysis leads to the accumulation of RNA in these neurons, while inhibition of the activity of neurons is accompanied by a decrease in the RNA content. Thus, the actual fact of the close relationship between the metabolism of nucleic acids in the neural tissue and the functional activity of the neural cells is not doubted. However, the internal mechanism of this relationship still remains unclear. Based on our own data from studying the motor neurons of the spinal cord (Brumberg, 1968) we can completely adhere to the conclusions of V. Ya. Brodskiy that were mainly made on the basis of an analysis of the neurons of the retina (Brodskiy and Pechayeva, 1958, 1959; Brodskiy, 1961, 1966) that the initial phase of intensified neuron activity is not accompanied by an accumulation of RNA. Apparently, the primary activation of the neuron is linked to some other mechanisms of the biochemical reconstruction of the nerve cell. Therefore in a comparative study of the RNA metabolism in the neurons and neuroglia we paid primary attention not to the direct reaction of neurons to the actual employed effect, but to the period of subsequent restoration of the normal state.

Previously Brumberg (1968) demonstrated that as a result of 3-hour swimming of the mice the RNA content was distinctly increased primarily in the spinal cord neurons, while their neuroglia did not display any significant shifts. Here both the behavior of the animals and the fact of the considerable increase in content of cytoplasmic RNA indicated that the
Figure 3. Changes in RNA Content in Neurons and Glia of Spinal Cord Anterior Horns of Mice after Cessation of 3-Week Hypodynamia
On horizontal--time (in h) after cessation of hypodynamia. Solid line--neurons, dotted--glia. Remaining designations the same as in fig. 1.

depletion of the main mass of motor neurons by the fourth hour had not yet occurred. Under these conditions the cessation of the load resulted, on the one hand, in a gradual normalization of the RNA content in the neurons, and on the other hand, in the appearance of distinct shifts in the content of glial RNA. This dynamics of the changes, according to the data of Brumberg (1968) was very diverse for the motor and sensory cells of the spinal cord. Significant differences between the motor and sensory sections of the spinal cord were also revealed during the restoration of the initial RNA level after cessation of the high motor activity of the rats induced by electrical skin stimulation of the animals (Pevzner and Khaydarliu, 1967; Khaydarliu, 1967a).

The results of this study are characterized by the fact that the dynamics of changes in the RNA content after cessation of hypodynamia was to a great extent very similar in the structures both of the spinal cord, anterior horns, and in the spinal ganglia (fig. 3 and 4). Apparently, such a similarity in the metabolic shifts in such functionally different neurons such as the motor neurons of the spinal cord and the sensory neurons of the spinal ganglia is due to the special nature of the studied effect. Hypodynamia for 2 and especially 3 weeks results, to all appearances, in adaptation, i.e., a state in which, in particular, catabolism and anabolism of RNA are well balanced with each other already on a new level corresponding to the conditions of hypodynamia. This is reflected in the absence of shifts in the RNA content in the motor and sensory neurons by the end of hypodynamia (fig. 1 and 2).
After the establishment of stable adaptation the transition of the animals to the conditions of free motor activity is for them strong, and apparently, nonspecific effect that sharply shifts the steady-state equilibrium in the RNA metabolism towards the dominance of catabolism. In fact, all the data of the physiological studies indicate that the most critical period from the viewpoint of the functional activity of the organism is precisely the transition from the lengthy forced hypodynamia to the state of normal mobility (Taranov and Panferonva, 1965; Kakurin et al., 1966; Katkovskiy, 1966; Mikhaylovskiy et al., 1967). This is also manifest biochemically in the distinct reduction in the RNA content both in the motor and the sensory structures of the spinal chord (fig. 3 and 4). Such an overexcitation of the neurons was, under our conditions, apparently more stable in the motor sections: the RNA content in the cytoplasm of the motor neurons of the anterior horns continued to drop even after 2 hours, reaching the minimum by 6 hours after cessation of hypodynamia, while in the neurons of the spinal ganglia in 6 h. restoration had already occurred of the normal RNA content. This corresponds to the data of Pevzner and Khandarliu (1967) that with the cessation of the electrical skin stimulation of the animals the rate of reparative changes in the content of cytoplasmic RNA was clearly higher in the motor neurons than in the neurons of the spinal ganglia. Thus, if the replacement of hypodynamia by rest in our previous works actually corresponded to the beginning of reparation in the cells of the nervous system, then cessation of the prolonged hypodynamia due to its peculiarities resulted in the fact that in the first hours
the free motor activity in itself was an extremely difficult process and essentially played the role of the next stress factor. Only further did true reparation occur, and in the rate of its occurrence biochemical differences already began to appear between the motor and sensory structures of the spinal cord.

Comparison of the dynamics of shifts in the RNA content in the neurons and neuroglia (fig. 3 and 4) demonstrates that both in the motor and the sensory sections of the spinal cord the glial cells were characterized as compared to the corresponding neurons by quantitatively sharper changes in the initial period after cessation of hypodynamia (first two hours), and further—higher rate of restoration. The initial RNA level in the cytoplasm of the motor neurons, for example, was restored only after 15-16 h, while in the body of the glial cells—already in 6 h at the end of hypodynamia (fig. 3). Analogous, although, less distinct temporary correlations were revealed also for cells of the spinal ganglia (fig. 4). Finally, an interesting although not yet explainable peculiarity of the glial cells was the repeated reduction in the RNA content in them in 3 days after the cessation of hypodynamia, i.e., in the period when the quantity of RNA in the neurons was returned to normal (fig. 3 and 4).

As a result of two-week hypodynamia the RNA content in the neuroglia of the spinal ganglia was clearly increased, after yet another week it was sharply reduced; in the neurons of the ganglia here there were no reliable changes in the RNA content (fig. 2). Currently we do not have any data to interpret such dynamics of the hypodynamic shifts in the content of glial RNA (the more so since analysis of the effect of the actual hypodynamia was not included in the direct task of this study). Possibly further with the accumulation of extensive information on the physiological and biochemical processes accompanying the forced hypodynamia the fact we revealed will receive the corresponding explanation.

On the whole, the obtained data confirm the previously advanced conclusion (Pevzner, 1965, 1967, 1968) that with sharp shifts in the functional state of the nervous system (for example, in anoxic hypoxia, pronounced
convulsions, prolonged electrical skin stimulation) changes in the RNA metabolism in the neurons and neuroglia can be directed in the same direction. Under conditions of the stimulation of the nervous system not resulting in its depletion or exhaustion, changes in the RNA content in the neural and glial cells can vary. The differences in the metabolism of neurons and neuroglia are clearly manifest also in the period of normalization upon cessation of the effect on the nervous system; in this period the neuroglia, as a rule, are characterized by the more rapid restoration of the previously altered RNA content. Probably such a high rate of the reparative processes guarantees the important compensatory, trophic role of the glial cells in the unified metabolic system neuron-neuroglia.

Conclusions

1. By the end of 2 and 3 weeks of hypodynamia induced by maintaining the mice in special chambers sharply restricting the movement of the animals no reliable changes were found in the RNA content in the cytoplasm of the motor neurons of the spinal cord anterior horns and in the body of the neuroglia cells surrounding them.

2. In the cytoplasm of the neurons the sensory spinal ganglia also did not reveal any significant changes in the RNA content by the end of 2 and 3 weeks of hypodynamia. In the glial cell-satellites of the ganglia the content of RNA was clearly increased by the end of the second and sharply reduced by the end of the third week of hypodynamia.

3. After the third week of hypodynamia in the first 2 hours after removal of the mice from the chambers the RNA content was reduced in the neurons and neuroglia both in the spinal cord anterior horns, and in the spinal ganglia. During the first days after the cessation of hypodynamia a gradual restoration of the initial RNA level occurred in the neural and glial cells (in a number of cases—with hypercompensation in the form of a temporary exceeding of this level); the rate of this restoration in the glia was higher than in the neurons. The dynamics of restoration of the RNA content in the cells of the anterior horns and the spinal ganglia somewhat differed.
4. In 3 days after cessation of hypodynamia both in the motor nuclei of the spinal cord and in the sensory spinal ganglia the RNA content in the neurons was normalized, and in the neuroglia was again reduced although to a lesser degree than in the first two hours after removal of the mice from the chambers.

5. Based on the comparison of the findings with the data of previous works of the authors differences are examined in the dynamics of reparative processes in the RNA metabolism in the system neuron-neuroglia after the cessation of hyper- and hypodynamia. The role is stressed of the neuroglia in the implementation of the compensatory, reparative, trophic processes in the nervous system, as well, possibly, in the adaptation on the cellular level.

References


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