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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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The vital activity of a living organism constantly proceeds under /1452* conditions of more or less motor activity, involving various physiological systems. The proprioceptive and interoceptive impulses of these systems toward the cells of the nervous system are one of the conditions of normal functioning of the neural fiber and consequently of the normal process of its intercellular metabolism. Therefore, the problem of forced hypodynamia has not only narrow applied and medical significance, but also general biological significance.

Substantial limitation of normal mobility over a prolonged period for both people and animals leads to a series of functional deviations on the part of the nervous system (Graveline et al., 1961; Gerd, 1963; Hatch et al., 1963; Van Reen, 1964; Gurvich and Efimenko, 1967; Krupina, et al., 1967). The question which concerns the extent to which these deviations affect the cellular metabolism in the nervous system is not investigated in detail.

Our experiment of quantitative cytochemical investigation of RNA in separate cells of the spinal cord under conditions of strenuous motor activity of a different source (Pevzner and Khaidarliu, 1967; Khaidarliu, 1967a; Brumberg, 1968) has confirmed the advisability of comparative studies according to physical differences of such objects as motor neurons of the ventral horns of the spinal cord and the sensory neurons of the spinal ganglia. In the presence of a series of characteristic distinctions in function, the embryogenesis and morpology of these kind of neurons are similar in the relationship that both these and

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*Numbers in the margin indicate pagination in the foreign text.

others have with the large mass of cytoplasm prevailing over the mass of the cellular nucleus (Khaidarliu, 1967b). This mass of cytoplasm is surrounded by glial cellular-satellites and enters into the composition of the spinal reflector arc, in which the neural ganglia play a role of afferent structure and the motor neurons of the ventral horns play the role of efferent structure. Therefore, although anatomically the spinal ganglia are not part of the spinal cord, we consider it possible to use them for comparison with the motor nuclei of the spinal cord and, conditionally, for the sake of brevity, we will call the sensory neurons of the spinal cord the neural ganglia.

The results of other experiments we have done (Pevzner, 1965, 1967, (illegible)), as well as the given literature (Hyden, 1962, 1964; Hyden and Lange, 1966) demonstrate the fact that the metabolism of the neurons and neuroglia may change differently under conditions of fluctuation of intensive functional activity of the nervous system. Less attention was specific to the comparison of the dynamics of the normalization process in neural and glial cells. Through the analysis of these dynamics, valuable information about the metabolic features /1453 of neuroglia may be given, which would provoke subsequent solutions to problems of the role of neuroglia in the functioning of neurons.

The problem of the present work was the comparison of the dynamics of the change in the RNA content-- an extremely important component of cells-- in the neurons and neuroglia of the ventral horns of the spinal cord and spinal ganglia under the influence of hypodynamia itself as well as, mainly, the subsequent normalization.

Methods

The experiments were carried out on male white mice weighing 28-32 g. Each animal was placed in a separate plexiglass compartment measuring 7.0 X 2.5 X 2.5 cm similar to the cages described by Fedorovii and Grishaninoi (1967). Such cages did limit the movement of the animals, but did not, however, lead to complete immobilization. The eating and drinking regimes of the experimental mice were kept the same as those of the control group.

The first few days after the mice were placed in the compartments they showed agitation, then gradually they became accustomed to the hypodynamic conditions and, in the long run, behaved quietly, sitting motionless in the compartments. Toward the end of 3 weeks of hypodynamia, a series of signs called a "hypokinetic complex" became noticeable in the animals: loss of body weight (about 15-20%), paresis of the dorsal end and loss of motor coordination. Normal motor activity in these mice was restored only after 2-3 days following the discontinuation of a 3 week hypodynamia.

After 2 and 3 weeks, part of the animals forgot the hypodynamia, another part, after a 3 week stay in the compartment were left in conditions of free motor activity and they forgot about the hypodynamia 2, 6, 24 and, 72 hours after being released from the compartment. They were then decapitated without anesthesia. The lumbar bulge of tne spinal cord with the spinal ganglia that border on it was fixed according to Brodsky's method in a cooled mixture of formaline, ethanol and acetic acid followed by a wash in paraffin. On the average thicknesses of 10 mm we determined the optical density of RNA in the phytoplasm of the neurons and the bodies of the glial cells up to and after the extraction of RNA to be 16% $NClO_4$ in the course of 8 hours. At temperatures of 0-4°C (Brumberg and Pevzner, 1966) the change in optical density was carried out with the help of two-wave variants (Agroskin et al., 1960) of ultraviolet exploration by ultraviolet cytospectrophotometry (Caspersson, 1936, 1950) in the presence of 256 and 280 nm on two-wave zones of ultraviolet cytospectrophotometry of Agroskin's construction; the drawing of the apparatus, details of the photometry and an account of the concentration of RNA were described earlier by Pevzner (1963, 1966). The quantity of RNA in the calculations on one cell were found as the product of the concentration of RNA on the volume of the cytoplasm of the neurons or the bodies of the glial cells. The volume was determined according to the formula of ellipsoid influence and linear measurements of the cells were obtained with the aid of a spiral ocular micrometer MOV 1-15X.

Each average increase in the RNA content was found according to the given photometry of 120-150 cells, taken from 5-7 animals. All

numerical material was worked out statistically by Student-Fisher.

Results

The determination of the quantity of RNA in the cells of the spinal cord was made after 2-3 weeks following the beginning of the hypodynamia, when, judging according to the animals' behavior, they had adapted enough to the living conditions of cramped quarters. The results of the investigation showed (figs. 1&2) that towards this time, actual changes in the cytoplasmic RNA content did not exist either in motor, or in sensory neurons. In the glial cells, bordering on the outer membrane of the motor neurons of the ventral horns, there was no essential change in the quantity of RNA (fig. 1). In the spinal ganglia, the glial cellular-satellites were characterized by a distinct increase in RNA content after 2 weeks and a decrease after 3 weeks following the beginning of hypodynamia. (fig. 2) /1454

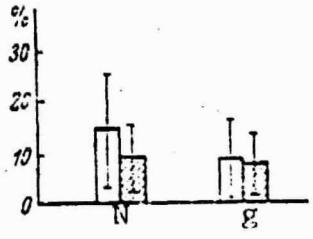

Fig. 1 The change in RNA content in motor neurons of the ventral horn of the spinal cord and the neuroglial cells surrounding them in mice after 2 and 3 weeks of hypodynamia.

On the vertical axis: the change in RNA content in the count of one cell (in % according to the comparison with the control group). N--neuron, g--glial. White column: 2 weeks of hypodynamia, slashed column: 3 weeks of hypodynamia. Vertical dash: doubled mean square errors.

The extraction of the animals from the compartments and the restoration of free motor activity were accompanied by a rapid, sharp increase of RNA content. As can be seen in figs. 3 and 4, already after 2 hours there was a remarkable decrease in the quantity of RNA in the neurons as well as in the glial cells of the ventral horns of the spinal cord an the spinal ganglia. With this, in both cases changes in the neuroglia were explained to a greater degree than changes in the neurons. The restoration was completed in the neuroglia earlier than in the neurons, after the restoration of the initial level of RNA in the spinal ganglia and in the features of the ventral horns of the spinal cord. In the long run, the RNA content in both kinds of cells was characterized from the start by an excess of the normal levels,

and then a small decrease in the neurons of the spinal cord (figs. 3&4). After 3 days following the discontinuation of the hypodynamia, the RNA content in the motor, as well as in the sensory neurons was practically normalized; in the neuroglia, the change (a decrease) in the quantity of RNA generally remains.

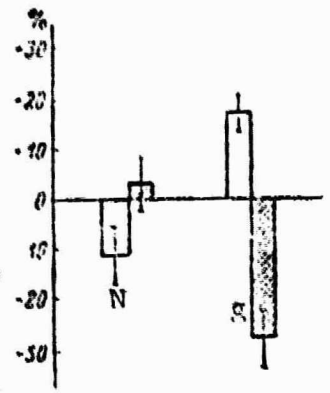

Fig. 2 The change in RNA content in neurons and neurglia of the sensory spinal ganglia in mice after 2 and 3 weeks of hypodynamia.

Scheme same as Fig. 1

Discussion

The multiplicity of cytochemical works, the average of which we chose to be the systematic investigations of Hyden and his colleagues in Switzerland (Hyden, 1962, 1964; Hyden and Lange, 1958, 1959) and Brodskii and his colleagues here (Brodskii, 1961, 1966; Brodskii and Nechaeva, 1958, 1959) have established that the stimulation of neurons still was not leading to their weariness or exhaustion. In the final analysis, stimulation leads to the accumulation of RNA in these neurons whereas the suppression of neural activity accompanied the decrease in the RNA content. In this manner, the simple fact that the metabolism of neuclitic acid in nervous tissue is closely connected with the functional activity of nerve cells is not doubtful. However, the internal mechanism of this connection still does not become completely clear. On the basis of substantial data, according to investigations of motor neurons in the spinal cord (Brumberg 1968), we may agree completely with the conclusions of V. Ya. Brodskii, mainly on the basis of an analysis of the neurons of the retina (Brodskii and Nechaeva, 1958, 1959; Brodskii, 1961, 1966) concerning the fact that

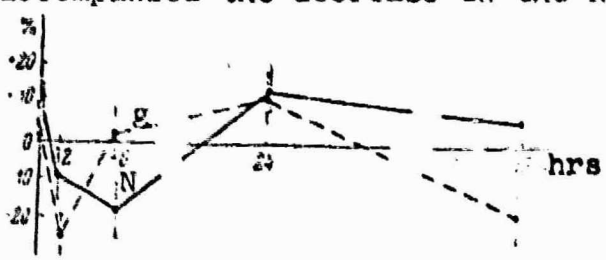

Fig. 3 The change in RNA content in neurons and glials of the ventral horns of the spinal cord in mice after the discontinuation of a 3 week hypodynamia.

On the horizontal: Time, in hours after the discontinuation of hypodynamia.
Solid line: neurons; dashed line: glials.

The rest of the scheme is the same as Fig. 1.

the beginning phase of strenuous activity of a neuron was not accompanied by an accumulation of RNA. Obviously the initial activity of the neuron is linked with the (illegible) mechanisms of biochemical reconstruction of nerve cells. /1455 Therefore, using a comparative study of the metabolism of RNA in neurons and neuroglia we gave basic attention not to an immediate reaction of the neurons at the application of the influence itself, but the period following the restoration of normal comportment.

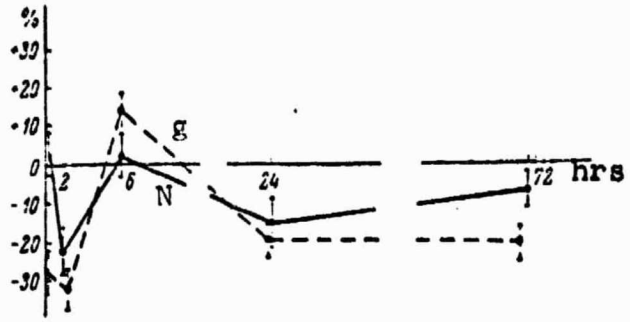

Fig. 4 The change in RNA content in the neurons and glials of the spinal ganglia in mice after the discontinuation of a 3 week hypodynamia.

Scheme same as Fig. 3.

Earlier, Brumberg (1968) had shown that, as a result of a mouse's 3 hour swim, the content of RNA distinctly increased, mainly in the neurons of the spinal cord, whereas in their neuroglia there was no notable substantial increase. Along with this, the behavior of the animals, as well as the fact of the remarkable increase in the content of cytoplasmic RNA, provides evidence for the fact that the weariness of the basic mass of motor neurons after about 4 hours still does not ensue. Under these conditions, the discontinuation of the load led to, from one part, the gradual normalization of RNA content in the neurons and from the other, it lead to the appearance of a distinct change in the glial RNA content. This dynamic change, according to Brumberg's (1968) data was extremely different for motor and sensory cells of the spinal column. The essential difference between the motor and sensory sections of the spinal column appears in the process of the restoration of initial levels of RNA after discontinuation of high motor activity of the rat, which was called electrocutaneous irritation of the animals (Pevzner and Khaidarliu, 1967; Khaidarliu, 1967a).

The results of the present investigation are characterized by the fact that the dynamics of the change in the quantity of RNA after the discontinuation of hypodynamia was much greater initially in the structures of the ventral horns of the spinal cord as well as in the

spinal ganglia (figs. 3&4). Obviously such a similarity of metabolic change in such physically different neurons as are motor neurons of the spinal cord and the sensory neurons of the spinal ganglia is stipulated by the special character of investigative influence. Hypodynamia in the course of 2 and, in particular,3 weeks leads, clearly, to adaptation, that is in the behavior, in the presence of which, in particular, the catabolism and anabolism of RNA is well balanced one with the other, already at the new level, corresponding to the conditions of hypodynamia. This is reflected in the absence of change in RNA content in motor and sensory neurons towards the end of hypodynamia (figs. 1&2).

After the establishment of degrees of adaptation the transfer of the animals to conditions of free motor activity shows, for them, a strong and obviously non-specific influence, a sharp movement establishing equilibrium in the RNA metabolism on the part of the predominance of catabolism. Of course, all data of physiological investigations are evidence for the most critical period from the point of view of functional activity of the organism namely, the transfer of prolonged forced hypodynamia to a state of normal mobility (Taranov and Panferova, 1965; Kakurin et al., 1966; Katkovskii, 1966; Mikhailovskii et al., 1967). This becomes apparent in biochemistry in a distinct drop in RNA content in motor as well as sensory structures in the spinal cord (figs. 3&4). Such re-stimulation of the neurons showed, in our conditions, obviously, in the more stable motor sections; the quantity of RNA in the cytoplasm of motor neurons of the ventral horn continued /1456 to drop and after 2 hours it reached a minimum at 6 hours after the the discontinuation of hypodynamia, whereas in the neurons of the spinal ganglia, after 6 hours the restoration of normal RNA content had already occurred. This corresponds to the data of Pevzner and Khaidarliu (1967) concerning the discontinuation of electrocutaneous irritation of animals where the rate of reparative changes in the cytoplasmic RNA content was in motor neurons, distinctly higher than in the neurons of the spinal ganglia. In this manner, if the change in hyperdynamic calm in our previous works was really corresponding to the beginning of the reparation in the cells of the nervous system, then discontinuation of prolonged hypodynamia in and of itself would lead to the fact that in the first hours, the freedom of motor activity appeared to be an

extremely difficult process and, according to the essence of the affair, played the role of investigating stress factors. Only in the long run does the true reparation ensue and at the rate that it proceeds, the biochemical differences between motor and sensory structures of the spinal cord are already apparent.

The comparison of dynamic changes of the quantity of RNA in the neurons and neuroglia (figs. 3&4) shows that in the motor and sensory sections of the spinal cord, the glial cells, characterized in comparison with the corresponding neurons are quantitatively more sharply changed at the beginning of the period after the discontinuation of hypodynamia (the first 2 hours) and in the long run, have a greater rate of recovery. The initial levels of RNA in the cytoplasm of the motor neurons, for example, were restored only after 15-16 hours, but in the fabric of the glial cells, initial levels were restored already after 6 hours following the end of hypodynamia (fig. 3). Analagous, though less clear cut, temporal correlations were shown for the cells of the spinal ganglia (fig. 4). Finally, it is interesting, though while not explained, to note the peculiarity of the glial cells was the repeated decrease in their RNA content after 3 days following the discontinuation of hypodynamia, that is, in the period when the quantity of RNA in the neurons returned to normal (figs. 3&4).

As a result of 2 week hypodynamia, the RNA content in the neuroglia of the spinal ganglia was distinctly raised, after one more week there was a sharp drop in the neural ganglia; in the presence of this, certain change in the quantity of RNA did not exist (fig. 2). At the present time we do not have any data for the interference of such dynamics of hypodynamic change in the glial RNA content (more than the fact that the analysis of the hypodynamia itself was not part of the immediate concern of the present investigation.) It is possible, in the long run, after the accumulation of more information about physiological and biochemical processes accompanying the forced hypodynamia, the fact we exposed will receive a corresponding explanation.

As a whole, the data received confirms the proposal (Devzner, 1965, 1967, 1968) mentioned earlier concerning the fact that, along with the sharp change in the functional make-up of the nervous system (for

example, anoxic (illegible), pronounced cramps, prolonged electrocutaneous irritation, changes of the metabolism of RNA in the neurons and neuroglia may be directed toward one and the same side.) Under conditions of stimulation of the nervous system not yet leading to its weariness or exhaustion, the change in RNA content in the nerve and glial cells may be different. The difference in the metabolism of neurons and neuroglia is distinctly shown and the normalization period of the neuroglia, as a rule, is characterized by a faster restoration of the quantity of RNA that had changed earlier. It is true that such a high rate of reparative processes provides an important compensatory trophic role of the glial cells in the only metabolic system of the neuron-- the neuroglia.

Conclusions /1457

1. At the end of 2 or 3 weeks of hypodynamia, the induced behavior of mice kept in special compartments with sharply restricted movement of the animals, a true change in RNA content was not found in the cytoplasm of the neurons in the ventral horns of the spinal cord or in the body of neuroglia surrounding these cells.

2. In the cytoplasm of the neurons of the sensory spinal ganglia, there was also no substantial change in the quantity of RNA towards the end of 2 and 3 weeks of hypodynamia. In the glial cellular-satellites of the ganglia, the RNA content was distinctly increased at the end of 2 weeks and sharply decreased at the end of 3 weeks of hypodynamia.

3. After a 3 week hypodynamia in the first 2 hours following the release of the mice from the compartments, the quantity of RNA decreased in the neurons and the neuroglia in the ventral horns of the spinal cord, as well as in the spinal ganglia. In the course of the first 24 hour period following the discontinuation of hypodynamia there was a gradual restoration of initial levels of RNA in the neural and glial cells (in a series of cases with a hypercompensation in the form of a temporary surpassing of this level); the rate of this restoration in the glials was higher than in the neurons. The dynamics of the

restoration of the quantity of RNA in the cells of the ventral horns and the spinal ganglia was a bit different.

4. After 3 days following the discontinuation of hypodynamia, RNA content was normalized in the motor nuclei of the spinal cord as well as in the sensory spinal ganglia, but in the neuroglia, it again decreased, though to a lesser degree than in the first 2 hours after taking the mice out of the compartments.

5. On the basis of a comparison of the results obtained with the data of previous work, the authors consider a distinction in the dynamics of reparative processes in the metabolism of RNA of the neural and neuroglial systems after the discontinuation of hyper- and hypodynamia. The role of the neuroglia in the realization of compensatory reparative and trophic processes in the nervous system must be emphasized, as well, possibly in adaptation at the cellular level.

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