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RNA CONTENT IN SPINAL CORD MOTONEURONS DURING HYPOKINESIA

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16. Abstract

The effect of a diminished motor activity of rats upon the RNA content in a single isolated motoneuron of frontal horns of their spinal cord was studied. Within a 1-30 days' exposure of rats to the hypokinetic conditions, RNA content was found to decrease on the 1st, 3rd and 5th day and to return to the initial level by the 7th day. No changes in RNA content were observed during the subsequent stages of the experiment. The volume of the nerve cells declined on the 3rd and 5th day, whereas RNA concentration reduced on the 1st, 3rd, 5th and 30th day.

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RNA CONTENT IN SPINAL CORD MOTONEURONS DURING HYPOKINESIA[1]

By

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One should now consider it established that a reduction in the motor function inevitably results in significant shifts in the metabolism of the organism of man and animals (Katkovskiy, 1966; 1967; Krupina et al., 1967; Portugalov et al., 1967; Fedorov, et al., 1968). There are data that the long stay of an organism in a state of hypokinesia is the cause of disorders in the nerve processes in man and animals (Gerd, 1963; Krupina, et al., 1967). In the literature one can also find information (Portugalov et al., 1967; Brumberg and Pevzner, 1968) on a disruption in cellular metabolism in the nervous system during restricted mobility. The purpose of this study was to investigate the effect of hypokinesia on the RNA content in motoneurons of the spinal cord anterior horns, since this test can serve as an indirect indicator of the state of nerve cell metabolism. /83**

Material and Technique

Experiments were conducted on 35 mongrel albino male rats weighing 260-300 g. The animals were placed in special, individual box cages of small size. The tails of the rats were attached by adhesive tape to a plastic dowel. This created conditions under which even the prolonged stay of the animals in the box cages did not result in the development of chafings, bedsores and broken skin.

[1] The article contains material prepared for candidate dissertation.

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

Thirty-five rats kept under vivarium conditions served as the control.

Spinal cord on the level of the intumescentia lumbalis was taken from the experimental and control animals simultaneously on the 1st, 3rd, 5th, 7th, 10th, 14th and 30th day of the experiment. Fragments of spinal cord 2-3 mm in size were fixed in Carnua solution for 1 hour and poured into paraffin. The motoneurons of the spinal cord anterior horns were isolated from sections 60 μ thick with the help of a Fonbryun micromanipulator under control of a MBI-3 microscope.

The RNA content in an individual, isolated cell was determined according to the method of Edstrom, (1964). For this the isolated cell (fig. 1, see insert VII)* was placed on a coverglass serving as the base of an oily chamber, and it was exposed to triple (40 minutes each at 37°C) effect of a ribonuclease solution (0.4 mg/ml) in 0.2 M of ammonia-bicarbonate-acetate buffer with pH 7.6. Then the extracts with the help of a micropipette made on a Fonbryun microforge were completely transferred to a quartz slide. After evaporation the dried extracts were dissolved in a microdrop of glycerin-phosphate buffer. The drops of solution under a layer of pentadecane (Maksimovskiy, 1969) were photometered on a recording ultraviolet microspectrophotometer MUF-5 by the scanning method with λ=257 mμ. The photometering was conducted with a 10 x lens and 0.07 mm probe. To calculate the quantity of RNA contained in the drop the optic densities of the palladium attenuators were recorded, and to determine the rate of movement of the table during the scanning the scale of the object-micrometer was recorded with λ=360 mμ. The quantity of RNA in an individual cell was computed according to the method of Slagel and Edstrom (1967) based on the similarity in the recording of the optic density of the drop to a parabola. Each average amount of RNA content in the cell was /84 determined from the results of analyzing 50-60 cells taken from 4-5 animals.

To calculate the RNA concentration in the cell the volume of the cellular body was determined from a formula for the rotation ellipsoid The largest and smallest diameters of the cell were measured with the help of a screw ocular-micrometer MOV1x15. Preliminary processing of the sections and isolation of the cells for size measurement were carried out according to the method described by Giacobini and Holmstedt (1958). Each average amount for the neuron volume was found from the data of measuring 100-120 cells taken from 4-5 animals. All the numerical material was processed by the method of variational statistics according to Student-Fisher.

*[Translator's note: insert not included in original.]

For cytochemical analysis the sections of spinal cord 7 μ thick were stained according to the method of Brashe and Eynarson.

Results and Discussion

Changes in the content and concentration of RNA were noted already on the first day after the beginning of the experiment. Both the concentration and the content of RNA in the nerve cells of the anterior horns of the experimental animals in the first five days of the experiment were lower than in the control animals (see fig. 2, a, c,; see table). Further, at later periods, all the way up to the 15th day, no changes were observed in the content and concentration of RNA in the motoneurons. On the 30th day of hypokinesia a distinct decrease in RNA concentration in the spinal cord motoneurons was again noticed.

Observation of the animals' behavior during the experiment showed that during hypokinesia two phases of motor activity are noted. The first phase lasts 1-5 days /85 and is characterized by the animals trying to free themselves from the unaccustomed conditions, which was accompanied in our experiments by an increase in motor activity. Further the animals seemingly became used to the new living conditions. The high motor activity of the animals in the period from 1 to 5 days of the experiment, apparently, can be evaluated as the cause of the drop in RNA content and concentration in the motoneurons of the spinal cord anterior horns and qualified as "the cytochemical equivalent of high functional stress and even overexcitation of these structures" (Portugalov et al., 1967). This is even more likely since there is published information on the link between the functional activity of the cell and the RNA content in it (Hyden, 1943; Hyden, 1963; Geynisman, 1965; Brodskiy, 1966).

Further, by the 7th day of the experiment, apparently, the animals adapt to the conditions of hypokinesia, i.e., a state occurs during which catabolism and anabolism of RNA are balanced well with each other; consequently, there are no changes observed in the RNA content and concentration. Staying under conditions of hypokinesia results in a decrease in the RNA concentration in the nerve cells by the 30th day of the experiment, although its content remains unchanged. The mechanisms for these phenomena have still not been disclosed.

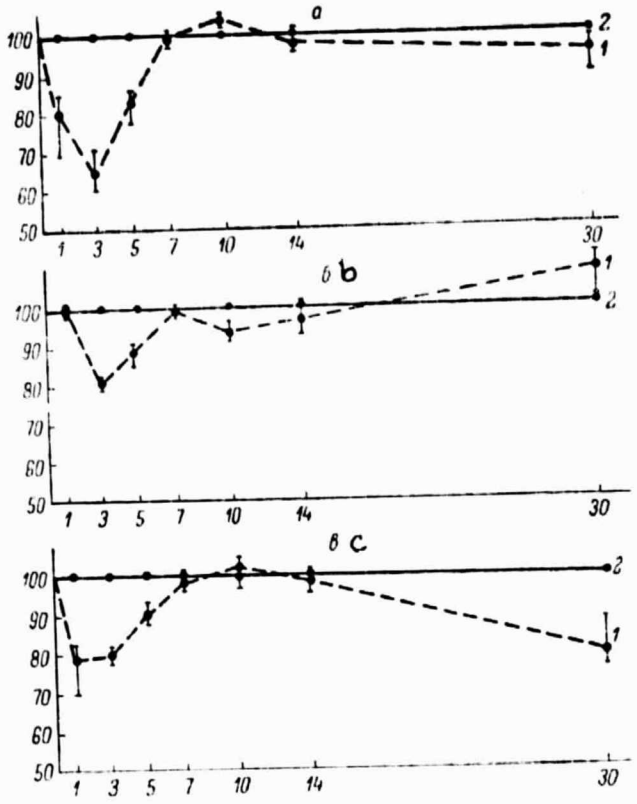

Figure 2. Change in Content, Concentration of RNA and Volume of Motoneuron of Anterior Horns of Spinal Cord in Rats at Different Periods of Hypokinesia

On x-axis--periods of hypokinesia (in days); on y-axis: a--RNA content (in %); b--volume of cell (in %); c--RNA concentration (in %); 1--experiment; 2--control

Parallel to a determination of the RNA content in the cell measurements were made of the volume of the motoneurons. On the first day of the experiment no reliable changes in the volume of neurons were obtained. On the third day of restricted mobility a distinct reduction was noted in the volume of the motoneurons that occurred up to the fifth day inclusively (fig. 2, b; see table). In the subsequent hypokinesia in the periods from 1 to 4 weeks (7, 10, 14, 30 days) there were no reliable shifts in the volume change (fig. 2, b; see table).

According to the data of a number of researchers (Borovskaya, 1935; Hyden, 1963; Zaguskin, 1964; Geynisman, 1965; Brodskiy, 1966; Khaydarliu, 1967) there is a clear correlation between the volume of the nerve cell body and its functional condition. However, the evaluation of the nature of changes in the volume of

CONTENT AND CONCENTRATION OF RNA IN MOTONEURONS OF SPINAL CORD ANTERIOR HORNS OF RATS IN DIFFERENT PERIODS OF HYPOKINESIA

Periods of hypokinesia (in days)	Variant	RNA content in cell (in μμg)	Volume of cell (in μ3 x 10-3)	RNA concentration in cell (in %)
1	Experiment	435 ± 36.7 (0.02 < P < 0.05)	20.5 ± 0.243 (> 0.05)	2.11 ± 0.15 (0.01)
	Control	541 ± 27	20.2 ± 0.124	2.67 ± 0.255
3	Experiment	383 ± 31.4 (0.002)	16.4 ± 0.468 (0.02 < P < 0.05)	2.126 ± 0.049 (0.001)
	Control	567 ± 35.1	20.2 ± 1.57	2.7 ± 0.0806
5	Experiment	427 ± 24.4 (0.05)	18.1 ± 0.61 (0.05)	2.5 ± 0.761 (0.02 < P < 0.05
	Control	509 ± 13.1	20.2 ± 0.875	2.5 ± 0.0663
7	Experiment	508 ± 1.42 (> 0.05)	17.1 ± 0.213 (> 0.05)	2.91 ± 0.387 (> 0.05)
	Control	515 ± 12.3	17.2 ± 0.751	2.94 ± 0.03
10	Experiment	462 ± 4.24 (> 0.05)	18.2 ± 0.304 (> 0.05)	2.4 ± 0.051 (> 0.05)
	Control	443 ± 5.04	19.25 ± 0.816	2.31 ± 0.043
14	Experiment	457 ± 19.3 (> 0.05)	21.2 ± 2.32 (> 0.05)	2.08 ± 0.03 (> 0.05)
	Control	465 ± 7.96	21.7 ± 2.45	2.09 ± 0.014
30	Experiment	450 ± 35.7 (> 0.05)	18.7 ± 0.673 (> 0.05)	2.413 ± 0.224 (0.05)
	Control	472 ± 29.2	17.2 ± 1.070	2.7 ± 0.0561

Note: The value of D is given in parentheses.

nerve tissue cells with a change in the functional state of the organism is still the object of discussion. Thus, some authors assume that no functionally-governed changes exist in the dimensions of the cell body (Hyden, 1943). The researchers who believe that this link does exist, cannot come to a unified opinion /86 on its nature. Some of them believe that the increase in the functional activity of the cell is invariably accompanied by an increase in its volume (Edstrom, 1957); others, on the contrary, believe that a reduction in volume accompanies an increase in function (Borovskaya, 1935). There is also the opinion that the increase in functional activity results in an increase in the volume of the cellular body, which is replaced by a reduction in volume as the cell becomes fatigued (Brodskiy, 1966; Geynisman, 1966). In our experiments, in the period of high motor activity of the animals, apparently, fatigue of the neurons occurred which, in all probability, also was the cause of a decrease in volume of the cellular bodies.

The reduction in volume of motoneurons on the 3rd and 5th day of restricted mobility can evidently be explained by the change in the distribution of water and ions, possibly associated with the change in the physicochemical properties of the membranes in the cellular structures (Brattgard et al., 1957) or change in the content of macromolecules, in the first place, cellular proteins (Hyden, 1943).

Changes in the RNA content in the cells can also be established with the use of cytological equipment (methods of Eynarson and Brashe). It follows from figure 3 (see insert VII)* that the stay of the animals under conditions of hypokinesia induces a reduction in the RNA content as compared to the control level in the motoneurons of the spinal cord on the 1st, 3rd and 5th days of the experiment.

Conclusions

1. When rats are kept under conditions of hypokinesia from 1 to 30 days a drop is established in the RNA content in the motoneurons of the spinal cord anterior horns on the 1st, 3rd and 5th day of the experiment, and its return to the initial level by the 7th day of the experiment. In the further periods of the experiment no changes were found in the RNA content.

2. A reduction in RNA concentration was found in the motoneurons of the spinal cord anterior horns on the 1st, 3rd, 5th and 30th days of the experiment.

3. On the 3rd-5th day of hypokinesia a reduction occurred in the volume of the nerve cells.

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