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THE INFLUENCE OF MODULATED SINUSOIDAL CURRENT ON THE STATE OF CHROMATIN FROM NEURONS OF THE CEREBRAL CORTEX OF RATS IN HYPOKINESIA

Translation of "Vliyanie sinusoidal'nykh modulirovannykh tokov ra sostoyanie khromatina neyronov kory golovnogo mozga pri gipokinezii", Voprosy Kurortologii, Fizioterapii i Lechebnoy Fizicheskoy Kul'tury, No. 1, Jan-Feb. 1978, pp 56-59

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16. Abstract

This article describes a study of the use of modulated sinusoidal current (MSC) to investigate the biochemical changes which appear under its influence in the central nervous system.

The methodology used is discussed with the results of the research presented in a table.

Three rather lengthy conclusions are presented.

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THE INFLUENCE OF MODULATED SINUSOIDAL CURRENT ON THE STATE OF CHROMATIN FROM NEURONS OF THE CEREBRAL CORTEX OF RATS IN HYPOKINESIA

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We have shown previously that the use of modulated sinusoidal current (MSC) in hypokinesia permits increased metabolism of nucleic acids in the skeletal muscles at which this stimulus is directed (Z.A. Sokolova, et al.). For a deeper study of the functional mechanism of MSC it is important to investigate the biochemical changes which appear under its influence in the central nervous system, particularly in individual nerve cells - the neurons of the cerebral cortex. Such investigation could enable one to establish the presence or absence of cellular specificity in the effect of MSC in hypokinesia, and clarify its effect on the metabolism of separate types of neurons of the cerebral cortex. */56

One of the basic criteria of the functional possibilities of any cell, in particular a nerve cell, is the degree of activation of its main genetic apparatus, chromatin. Therefore our problem includes the study of the effect of MSC and measured physical stress on the structural and functional state of nuclear chromatin from various types of neurons of the cortex of the large brain hemispheres of rats in hypokinesia.

The work was carried out on 50 white male rats of the Vistar strain weighing 160-200 g, divided into five groups of 10 animals each. The first group consisted of intact rats; group 2 of rats kept in hypodynamia for 30 days; group 3 of healthy rats subjected to measured physical stress; group 4 of rats subjected to MSC during a period of immobilization; and group 5 consisted of rats subjected to measured physical strain during a period of hypokinesia. Hypokinesia was obtained by holding the rats in individual "hypokinetic" cages

*numbers in margin indicate pagination of foreign text.

which sharply limited the animals' mobility. For the MSC stimulus an "Amplipulse-3" apparatus was used (carrying current frequency 5000 CPS, modulation depth 100%, frequency 100 CPS, operation type 2, current strength 1.5-2.5 milliamperes). We carried out a procedure lasting 20 minutes twice daily for 20 days. The stimulus was applied to the region of both haunches. Running a treadmill for 20 minutes daily for 20 days served as measured physical stress (the treadmill track moved at a constant linear speed of 20 cm/sec).

Chromatin from the nuclei of cerebral cortex cells was examined by Rigler's microflucrometric method, the basis of which is measurement of the intensity of fluorescence of the complex of acridine orange (AO) and DNA chromatin. Intensity of fluorescence depends on the quantity of AO, bonded to DNA included in the composition of desoxyribonucleoprotein (DNP), and reflects the mutuality of the DNA-protein system. The method provides an idea of the degree to which the DNA chromatin molecule is blocked by proteins, and consequently of the degree of its matrix activity (Rigler et al; Ringertz).

The intensity of fluorescence was measured in individual cells of the large pyramidal neurons of layer V and the stellate neurons of layer IV from the kinesthetic zone of the cerebral cortex (the classification of V.M. Svetukhina was used in determining zones) and also in the granule neurons of the dentate fascia of the hippocampus. For this brain tissue was fixed for 30 minutes in a mixture of equal quantities of ethyl alcohol and acetone at room temperature, then for 20 hours in the same fixative at a temperature of 4°C. From the paraffin blocks prepared by the commonly accepted method, sections of respective parts of the brain 5 micrometers in thickness were obtained. /57
After preliminary deparaffination the preparates were processed according to Rigler's method, stained with an AO solution, prepared on a citrate-phosphate buffer pH 4.1. The

quantity of AO combining with the nucleus was determined according to the intensity of fluorescence at a wavelength of 530 nm (F_{530}), expressed in conventional units. In each preparate measurements were made of not fewer than 10 nuclei of corresponding types of nerve cells on a luminescent MSP-0.5 microscope-photometer from the "Opton" firm.

test conditions		large pyramidal neurons	stellate neurons	granule neurons of the dentate fascia of the Hippocampus
Norm (control)		11,2±1,02	5,8±0,87	7,2±1,32
physical stress		13,6±1,99	5,3±0,51	6,8±1,24
	P	>0,25	>0,5	>0,5
hypokinesia		7,2±0,99	3,8±0,33	6,2±0,86
	P	<0,002	<0,05	>0,5
hypokinesia + SMC		13,6±1,40	5,1±0,99	6,8±1,45
	P	<0,001	>0,1	>0,5
hypokinesia + physical stress		8,6±1,40	4,6±0,41	6,6±0,89
	P	>0,25	>0,1	>0,5

Table. Intensity of fluorescence of DNP (F_{530}, in conventional units) from nuclei of neurons from cerebral cortex of rats in control, with hypokinesia, effect of MSC and measured physical strain (M±m)

The results of the research are presented in the table. Simultaneous examination in the same preparations of large pyramidal and stellate neurons from the kinesthetic zone of the cerebral cortex permit one to make a comparative analysis of the intensity of fluorescence in the nuclei of these cells in intact rats. It showed that intensity of fluorescence in

intact nuclei of large pyramidal neurons is almost twice as great as that in stellate neurons. These data permit one to consider that in the control the genetic apparatus of large pyramidal neurons in comparison with stellate neurons is less repressed by proteins, and consequently is in a more active condition.

Research on rats kept in a regime with sharply limited motor activity permitted us to establish that intensity of fluorescence in the large pyramidal and stellate neurons of the cerebral cortex in these animals compared with intact animals was significantly decreased. With this the ability of DNA chromatin to bind AO showed statistically reliable decrease in the nuclei of large pyramidal neurons by 35.7% ($P<0.02$) and in stellate neurons by 33.5% ($P<0.05$). Intensity of fluorescence in nuclei of granule cells of the dentate fascia of the hippocampus in hypokinetic rats corresponded to its degree in intact animals.

As opposed to hypokinesia, physical stress applied to healthy rats did not noticeably influence the stain-binding property of CNP from large pyramidal and stellate neurons of the cerebral cortex or of granule cells of the dentate fascia of the hippocampus.

Under the influence of MSC, to the effect of which rats were subjected while immobilized, significant changes in the indices under study took place only in the nuclei of the large pyramidal neurons; the intensity of their luminescence reliably exceeded 89% ($P<0.001$).

The ability of DNP from stellate neurons to bind AO also somewhat increased with the effect of MSC; however, this increase was not statistically significant. Adsorption of AO by the nuclei of granule cells of the hippocampus in these experimental conditions remained practically unchanged.

Physical stress applied to rats during hypokinesia did not lead to significant changes in the intensity of fluorescence in DNP of nuclei of the types of neurons examined; only a tendency to increase was observed in nuclei of large pyramidal and stellate neurons. /58

Results of our research showed that both the state of hypokinesis and the effect of MSC have a definite effect on the structural properties of chromatin from the nuclei of neurons of the cerebral cortex of rats. The changes arising in these circumstances are ambiguous in different experimental conditions, and show specificity in different types of nerve cells.

In studies carried out on solutions of DNP and chromatin from different types of cells (hepatocytes, lymphocytes from peripheral blood), a relationship was established between the intensity of fluorescence in DNP and the quantity of protein in reaction with DNA in the desoxyribonucleoprotein complex (O.F. Borisova and E.S. Minyat; K.N. Fedorova). Such structural changes of the relationship between DNA and protein in DNP composition are regarded as closely linked with changes in the fundamental properties of chromatin and its matrix activity (A.Ya. Varshavskiy).

The decreased intensity of fluorescence in nuclei of large pyramidal and stellate neurons with hypokinesia indicate that the genetic apparatus of these nerve cells is evidently more largely repressed by proteins than in intact rats, and consequently its matrix activity is also decreased.

Application of MSC in a period of hypokinesia leads to increased adsorption of AO by DNA chromatin of large pyramidal neurons, and greater lability of the bond between DNA and proteins or their partial detachment from the DNA molecule.

Considering the data presented from the literature, such conformational changes in chromatin DNA, arising under the influence of SMC, may be regarded as activation of the genetic apparatus of these neurons. The properties of DNP of nuclei from stellate neurons and granule cells of the dentate fascia of the hippocampus did not change upon application of MSC. These data permit one to conclude the presence of cellular specificity in the functional mechanism of MSC with hypokinesia.

Physical stress applied both to intact and to hypokineticized rats did not significantly affect the state of chromatin in the type of cerebral cortex neurons studied. Evidently the genetic apparatus of neurons of the cerebral cortex in the process of phylogenesis adequately adapted to the effect of a factor which by its nature may be classed among natural biological stimuli.

The results obtained also permit one to state that the different types of neurons studied react variously to the same stimulus. The greatest reactivity was shown by nuclei of large pyramidal neurons, the metabolism of which according to the indices of intensity of fluorescnece of DNP changed both under hypokinesia, and upon the effect of MSC. The degree of activiation of the genetic apparatus of stellate neurons changed only in hypokinesia. Most stable were found to be the properties of chromatin from granule neurons of the dentate fascia of the hippocampus; the intensity of the fluorescence of its nuclei did not change in reaction to either applied stimulus. The different degree of activation of the genetic apparatus of the studied nerve cells of the cerebral cortex in intact rats, and also the fact that one and the same stimulus was accompanied by ambiguous shifts in the metabolism of these cells, indicate that different types of neurons possess different structural and functional genome organization. One may suggest that these structural and functional differences lie at

the basis of the specific activity of the examined types of neurons from the cortex of the large brain hemispheres.

Conclusions /59

1. Immobilization of rats for 30 days in "hypokinetic" cages is accompanied by decreased intensity of fluorescence in the DNA of chromatin from nuclei of large pyramidal and stellate neurons of the kinesthetic zone of the cerebral cortex. Adsorption of acridine orange by the nuclei of granule cells from the dentate fascia of the hippocampus did not change under these conditions.

2. Sinusoidal modulated current, to the action of which rats were subjected in a condition of hypokinesia, provoked a statistically reliable increase in the intensity of DNP fluorescence in large pyramidal neurons, which indicates activation of the genetic apparatus of these nerve cells, and did not have any marked effect on the ability of the nuclei of stellate neurons and granule neurons of the dentate fascia of the hippocampus to bind the stain.

3. Measured physical stress did not affect the stain-binding property of DNP from the studied types of cells in either or hypokinetic rats.

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