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TOPOCHEMICAL DIFFERENCES IN THE AMOUNT OF RNA IN THE MOTONEURONS OF THE SPINAL CHORD IN HYPOXIA AND HYPOKINESIA

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16. Abstract Reactions to hypoxia and hypoknesia are compared by measuring changes in the amount of RNA in the cytoplasm of neurons of the intumescentia cervicalis and lumbalis. Animals were subjected to hypoxia, hypokinesia and both combined and a control group to neither. 2 groups of motoneurons compared - one innervating the respiratory musculature, the other the musculature of the lower extremities, so that hypoxic hypoxia would probably affect the first group primarily and hypokinesia the second. Neither affect the amount of RNA in the neurons of the first group but a significant increase is noted in neurons of the second group. Hypoxia is normal to rats and their compensatory mechanism well developed but prolonged hypokinesia is not, so that the shift in the amount of RNA in the neurons of the intumescentia lumbalis during hypokinesia was twice as marked as during hypoxia. Combined hypoxia and hypokinesia weakened the effect of hypokinesia alone. The increase in the amount of RNA probably reflects a lowering of the energy consumption of cellular protein and thus also a decrease in the requirements for RNA, since a certain inhibition of metabolism should occur.

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TOPOCHEMICAL DIFFERENCES IN THE AMOUNT OF RNA IN THE MOTONEURONS OF THE SPINAL CHORD IN HYPOXIA AND HYPOKINESIA

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It was shown earlier [1], that prolonged hypoxic hypoxia induced in rats when /1490* kept in a decompression chamber leads to a non-uniform picture of changes in RNA content in the cytoplasm of neurons as a function not only of the hypoxia regime but also of the type of neuron. A comparison was made of Purkinje cells in the cerebellum and motoneurons of the spinal chord, neurons very essentially different in their morphological characteristics, functional activity and specific neuromediators. Differences have been established for these two types of neurons in respect to shifts in cytoplasmic RNA content as well as for various other experimental effects [2-8].

In the present work a comparison was made of reactions to hypoxia and hypokinesia on the part of the motoneurons of the cervicle and lumbar intumescence of the spinal chord, i. e. neurons that are similar morphologically and in the part played by the neuromediator in their functional activity but different in the muscle groups they innervate. In particular, a very important circumstance is the fact, that the motoneurons of the cervical intumescence innervate the diaphragm and intercostal muscles that play a basic role in the regulation of respiration. These two conditions are essentially differentiated by hyperventilation in hypoxia and a certain reduction in ventilation in hypokinesia.

The experiment was conducted on sexually mature male rats weighing 100±20 g. They were divided into 4 groups. The rats in group I were placed in the decompression chamber at first at an "altitude" of 3,000 m, which was raised daily for a week by 500-1000 m , and then at an "altitude" of 7,000 m which was maintained for 14 days. Daily exposure of the rats to these rarefied atmospheric conditions was 6 hours. According to the data of Vasil'yev et al. [9], such a training program produces a high degree of adaptation. The rats in group II were subjected to prolonged forced hypo-

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

kinesia by being placed for 20 days in small box cages that significantly restricted the scope of possible movements. Finally, group III were subjected to the combined effect of hypoxia and hypokinesia by being confined in box cages and placed in the decompression chamber and maintained under the same hypoxia conditions as the rats of group I. An added control was furnished by rats of group IV kept in simple cages under normal atmospheric pressure. Each group contained 5 animals and 25-30 cells were taken from each animal.

At the end of the experiment the rats were decapitated in the absence of anesthesia and specimens of the spinal chord taken from the regions of the cervical and lumbar intumescence were fixed in a Brodski mixture of formalin, ethanol and acetic acid and poured in paraffin. Sections 7-8 microns thick were treated for 8 minutes with 5% trichloroacetic acid at 90°. Before and after extraction the motoneuron cytoplasm samples were subjected to UV cytospectrophotometry at 265 and 280 mμ on a probing double frequency Agroskin cytospectrophotometer. The arrangement of the cytophotometer and the method of using double frequency cytospectrophotometry on cellular RNA of the nervous system has been described previously [10-12].

TABLE 1. CONCENTRATION OF CYTOPLASMATIC RNA, AMOUNT OF CYTOPLASM AND ABSOLUTE AMOUNT OF CYTOPLASMATIC RNA IN MOTONEURONS OF THE ANTERIOR HORNS OF THE CERVICAL AND LUMBAR INTUMESCENCE OF THE SPINAL CHORD IN HYPOXIA AND HYPOKINESIA *.

a Отдел спинного мозга и условия опытов	b Концентрация РНК, %	c Объем цитоплазмы нейронов, μ^3	d Количество РНК (в пг) в расчете на 1 клетку
Контроль e	3,7±0,1	6 780±380	260±17
	3,1±0,1	12 200±690	380±27
Гипоксия f	3,7±0,1	7 060±380	265±17
	3,9±0,1 **	12 420±870	480±39 ***
Гипокинезия g	3,3±0,1***	8 100±530	260±20
	4,4±0,1 **	11 700±890***	525±50 **
Гипоксия + гипокинезия h	3,6±0,1***	8 640±630	310±26
	4,1±0,1 **	12 330±730	510±34 **

*Figures above the line - data for cervical intumescence, below - for lumbar.
**Statistically reliable difference from control at $P < 0.01$.
***The same at $P \leq 0.01$

Key: a. Region of spinal chord and experimental conditions.
b. Concentration of RNA in %.
c. Amount of cytoplasm of neurons, μ^3.
d. Quantity of RNA (in pg) calculated for a single cell.
e. Control.
f. Hypoxia.
g. Hypokinesia
h. Hypoxia + hypokinesia.

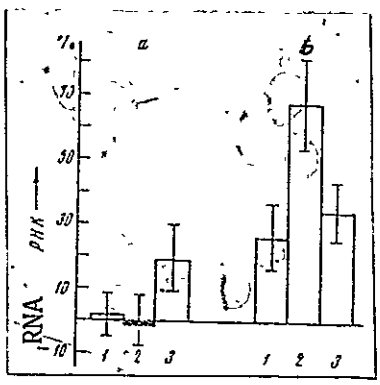

Fig. 1. Relative changes in the amount of cytoplasmic RNA in the motoneurons of the anterior horns of the (a) cervical and (b) lumbar intumescence of rat spines under hypokinesia and hypoxia. 1 - hypoxic hypoxia; 2 - forced hypokinesia; 3 - combined hypoxia and hypokinesia.

The data presented in Table 1 indicate, that /1491 the concentration of cytoplasmatic RNA in the motoneurons of the spinal chord is definitely reduced only under conditions of hypokinesia, while in the motoneurons of the lumbar intumescence RNA concentration appeared distinctly higher when compared with the control in all the experimental series. Hypokinesia produced a clear increase likewise in the amount of cytoplasm in the motoneurons of both spinal regions, whereas combined hypoxia and hypokinesia induced an analogous increase only in the neurons of the cervical intumescence. As a result, the absolute quantity of cytoplasmatic RNA (calculated for a single cell) in the neurons of the cervical portion under the influence of hypoxia alone as well as of hypokinesia alone remained unaltered. Their combined effect produced some accumulation of RNA in the cytoplasm of the motoneurons. The quantity of RNA in the cytoplasm of the motoneurons of the lumbar intumescence of the spinal chord rose distinctly following training in the decompression chamber and the rise was particularly sharp as the result of hypokinesia. A combination of hypoxia training and hypokinesia produced substantially the same effect as hypoxia training alone.

Thus under identical conditions RNA metabolism in the motoneurons of different sections of the spinal chord did not change in a uniform way. In the neurons of the cervical region the absolute quantity of cytoplasmatic RNA appeared to be significantly more stable: it appeared only under combined hypoxia and hypokinesia (Fig. 1a). However in the neurons of the lumbar intumescence all active intervention produced a distinct increase in the amount of cytoplasmatic RNA (Fig. 1b).

Acute hypoxic hypoxia induces an accumulation of cytoplasmatic RNA in the motoneurons of the spinal chord [1,2,7]. However, repeated activity (hypoxia training) probably activates numerous compensatory mechanisms as the result of which, particularly, there is a restructuring of the intracellular metabolism of RNA as well in the neurons of the spinal chord, setting at a new level the interplay of its synthesis and disintegration. This seems to explain the absence of an increase in RNA con-

tent in prolonged adaptive action of hypoxia [1]. There is no doubt, that under conditions of hypoxic hypoxia the motoneurons of the cervical intumescence experience a much more intense physical load than the lumbar, since it is precisely the cervical /1492 motoneurons that take care of such an important compensatory reaction component of the organism to hypoxia as hyperpnea.

Prolonged forced restriction of motor activity (hypokinesia) also produces its own type of stress. In a study of the dynamics of RNA content in the motoneurons of rat spines over a 3 week period of hypokinesia Gorbunova [13] showed, that a distinct change in RNA content set in only in the course of the first week of hypokinesia and thereafter the amount of RNA in the motoneurons persisted at the normal level. According to the data of Brumberg and Pevzner [14] working with mice, at the end of the 2nd and 3rd week of hypokinesia the amount of cytoplasmatic RNA in the motoneurons of the spinal chord was also normal; however the moment the animals were permitted to move freely after the 3rd week of hypokinesia the amount of RNA in the cytoplasm of these neurons clearly went down. We may suppose, that under conditions of prolonged hypokinesia an equilibrium is set up in the motoneurons between RNA anabolism and catabolism which corresponds to the altered conditions of function in these cells. Undoubtedly the point at which this equilibrium is set up is determined by the totality of the characteristics of the hypokinetic regime and of the functional qualities of concrete neurons. As is seen in Fig. 1, at the end of 20 day hypokinesia changes in RNA content were noted only in motoneurons of the lumbar intumescence in the absence of positive shifts in the cervical. It is hard to imagine, that such non-physiological conditions as severe restriction of mobility for 3 weeks would have no effect on RNA metabolism in motoneurons. Probably restriction of movement in the upper extremities and diminution of respiratory excursions of the thorax in animals like rats comprises a less traumatic factor than restricted mobility of the powerful lower limbs. We may suppose, that the restructuring of RNA metabolism in the neurons of the cervical intumescence, which innervate the musculature of the upper extremities and the diaphragm, is more easily realized than that of metabolism in the neurons of the lumbar intumescence.

The data obtained make it possible to draw some conclusions about the different nature of hypoxic and hypokinetic types of stress. We compared two groups of motoneurons, one of which innervates the respiratory musculature and the other the musculature of the lower extremities. We may suppose, that hypoxic hypoxia would have an

effect on the first group preferentially and hypokinesia on the second group of neurons. Meanwhile a comparison of the data obtained (Fig. 1) indicates, that neither of these effects alters the amount of RNA in neurons of the first group and yet they both have a similar influence (increase) on the RNA content in neurons of the second group. Apparently, from the ecological point of view hypoxia is a stress factor frequently met in the daily life of rats and consequently the antihypoxic compensatory mechanisms of these animals are rather well developed. Prolonged hypoxia for rats, that are mobile and non-hibernating animals, is ecologically a very unusual factor. /1493 Thus it does not seem fortuitous, that a displacement of RNA in the neurons of the lumbar intumescence due to hypokinesia appeared to be almost twice more marked than that due to hypoxia (Fig. 1b). At the same time our attention is drawn to the fact, that combined hypokinesia and hypoxia attenuated the effect of hypokinesia alone.

The increase in the RNA content in the motoneurons which we noted in hypokinesia in this experiment seems to be expressive of a drop in cellular protein expenditure and, linked with it, a decreased demand for RNA, since under the given conditions there has to be some inhibition of metabolism.

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