reaction of a cellular apparatus producing excess of Fn, in accordance with the feedback principle. The increased biosynthetic activity of CDF in OI is evidence in support of this suggestion.

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EFFECT OF DIFFERENT HYPERBARIC OXYGENATION SCHEDULES ON TRANSCRIPTION ACTIVITY AND MORPHOLOGY OF FRONTAL CORTICAL NEURONS IN RATS WITH OCCLUSION OF THE COMMON CAROTID ARTERY

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KEY WORDS: hyperbaric oxygenation; neurons; ischemia; transcription; morphology

The problem of optimization of therapeutic schedules of hyperbaric oxygenation (HBO) for patients with stroke is due partly to the complexity of the pathogenesis of cerebral ischemia and partly to inconstancy of the action of different HBO schedules. A comparative study of the efficacy of different HBO schedules for the treatment of stroke has been undertaken, but only fragmentarily, with no attempt to analyze small doses of hyperoxia such as we ourselves have developed [3]. The discrepancy between the results of clinical and experimental studies of the efficacy of HBO in the treatment of stroke and the absence of any research into optimization of HBO schedules depending on the severity and duration of cerebral ischemia necessitated an investigation such as that described below [7-9, 11, 12].

EXPERIMENTAL METHOD

To study the mechanisms of action of different HBO schedules in the treatment of cerebral ischemia a combined study was undertaken on noninbred male albino rats weighing 200 g with unilateral and bilateral ligation of the common carotid arteries below the thyroid cartilage under pentobarbital anesthesia (0.02 g/100 g body weight). This communication describes the results of an investigation of transcription activity and morphology of frontal cortical neurons after occlusion of the right common carotid artery, accompanied by early (3 h of ischemia) and late

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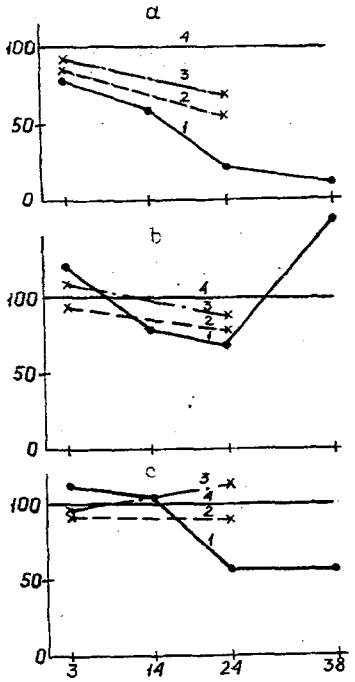

Fig. 1

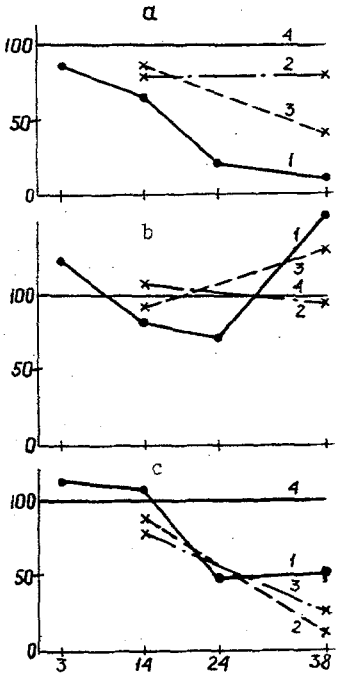

Fig. 2

Fig. 1. Time course of morphological and functional parameters of large and medium-sized frontal cortical pyramidal neurons in rats with unilateral occlusion of common carotid artery treated early with HBO: a) morphological integrity. Abscissa, time after session (in h). Ordinate, fraction of intact neurons (in % of control); b) template activity of nucleolar chromatin. Abscissa, time after session (in h). Ordinate, change in labeling level (in % of control); c) template activity of extranucleolar chromatin. Abscissa, time after session (in h); ordinate, change in labeling level (in % of control).

Fig. 2. Time course of morphological and functional parameters of large and medium-sized frontal cortical pyramidal neurons in rats with unilateral occlusion of common carotid artery treated late with HBO. Filled circles denote points at which mean level of parameters studied during ischemia without HBO differed significantly in Wilcoxon's test from control; crosses indicate points at which mean level of parameters studied during ischemia treated by HBO differed significantly by Wilcoxon's test from that without HBO.

(14 h after occlusion) single exposure to two HBO schedules, namely 2.0 atm with exposure for 1 h and 1.2 atm with exposure for 30 min. The animals were killed 15 min and 1 h after HBO. Animals undergoing the full operation or a mock operation, but not receiving HBO, and intact rats either receiving or not receiving HBO served as the controls. Each comparison group consisted of three animals.

Several parallel investigations were carried out on material taken at the same time: a general morphological analysis of frontal cortical preparations stained by Nissl's method (to evaluate the state of the large and medium-sized pyramidal neurons – the average percentage of relatively intact cells in the visual field, and also the number of reactively and pathologically changed neurons). At the same time functional activity of the cell nucleus was assessed, with special reference to transcription activity of nucleolar and extranucleolar chromatin of the corresponding neurons. To assess transcription activity of chromatin, the histoautoradiographic method suggested in 1978 by Moore [10] was used. Its unique feature is that the RNA-polymerase reaction is carried out in situ, with participation of endogenous RNA-polymerases, actually on the histological section of the test tissue. The level of transcription

activity of the nuclear structures of the residual neurons was assessed as the number of grains of reduced silver above them, for nucleolus and nucleoplasm separately. The significance of differences between the experimental groups was estimated by Wilcoxon's test or the φ test.

EXPERIMENTAL RESULTS

On the whole the general morphological changes in the frontal cortex of animals with carotid arterial occlusion corresponded to the description given by Bogolepov [1], depending on the severity and time of ischemia. In animals undergoing the mock operation, general morphological investigations revealed no appreciable differences from the control in the large and medium-sized pyramidal neurons.

In animals with carotid occlusion and not treated by HBO, the number of intact neurons in a field of vision was 20% lower than in the control as early as 3 h after the operation (possibly on account of edema also). The number of intact pyramidal cells 14 h after the operation was reduced to 60% of the control value and the residual neurons showed more marked morphological changes. Cells with a convoluted nuclear membrane, with a reduced nucleocytoplasmic ratio, and with hypertrophied nucleoli at this time accounted for 50% of the residual subpopulation. There were many cells with total chromatolysis and many vacuolated neurons (Fig. 1a).

The morphological picture 24 h after the operation showed worsening: the fraction of relatively intact pyramidal cells was only about 20% of the control value, and the cells were mainly medium-sized and in a state of total chromatolysis, or neurons with a lowered nucleocytoplasmic ratio; they were often hyperchromic, had lost their pyramidal shape, and had shrunken nuclei and enormous nucleoli. This period of ischemia was characterized by the appearance of neuronophagy.

The fraction of relatively intact cells 38 h after the operation, in rats not receiving HBO, did not exceed 10% of the control and there were many zones of necrosis where practically no cells remained intact.

A very small increase in transcription of the intact neurons in the early stages of ischemia was followed by marked inhibition, which for the extranucleolar chromatin corresponded to about 50% of the control after 24 and 38 h (Fig. 1b). At the same time, the significant decrease in nucleolar transcription observed 14 h, and progressing 24 h after the operation, was replaced by marked activation of transcription after 38 h (Fig. 1c).

The use of HBO in the early stage of ischemia (3 h) was accompanied by normalization of the morphological picture of the frontal cortex when investigated immediately after the session. A somewhat higher percentage of neurons in the field of vision was observed after HBO at 2.0 atm (possibly on account of more marked dehydration), 24 h after the HBO session (Fig. 1a).

The effect of early administration of HBO on nucleolar and nucleoplasmic transcription is shown in Fig. 1b, c. The early use of HBO normalized the average parameters of transcription in the presence of cerebral ischemia. No reduction of nucleoplasmic transcription in the intact neurons had developed 24 h after HBO. Moreover, HBO at 2.08 atm actually led to significant activation of nucleoplasmic transcription compared with the control (Fig. 1c).

With late treatment by HBO, i.e., 14 h after the operation (Fig. 2), appreciable normalization of the morphological changes in the frontal cortex was observed immediately after the session, the percentage of intact cells rose to 80%, the normal nucleocytoplasmic ratio was restored, and hyperchromatosis and other changes in the neurons were less marked; the effect, moreover, was independent of the dose of HBO. However, 24 h after the session, significantly better preservation of the neurons was observed in the group of animals receiving HBO at 1.2 atm (Fig. 2a). In animals receiving HBO at 1.2 atm the morphological state of the large and medium-sized pyramidal cells after 24 h was similar to that found immediately after the session. In other words, this dose of HBO apparently stabilized the situation, and prevented progression of the pathological changes in the cells studied. The protective effect of a dose of 2.0 atm was less marked, but in this case also HBO had a definite stabilizing action. The morphological picture of the frontal cortex 24 h after HBO at 2.0 atm was similar to that described for the group of animals not receiving HBO, 14 h after the operation.

The effect of a late HBO session on transcription of nucleolar genes of the neurons studied immediately and 24 h after the session was normalizing in character, and was more marked in the case of 1.2 atm (Fig. 2b). As regards the transcription activity of the extranucleolar chromatin, late application of HBO, irrespective of the dose given and the time elapsing after the session, had a suppressive action, and aggravated the inhibitory effect of ischemia in the late stages after the operation (Fig. 2c).

The investigation demonstrated the marked protective action of HBO relative to morphological preservation of cortical pyramidal neurons in the zone of developing ischemia. Definite dependence of the effect on dose was observed in only one case, namely 24 h after a late session. The inverse character of this dependence may perhaps be connected with the spastic effect of a dose of 2 atm on the cerebral arterioles and capillaries, which we found in the course of an electron-microscopic investIgation [4]. The action of HBO on transcription demonstrates a somewhat complex dependence on the conditions of HBO. Unlike an early session, the effect of late treatment is normalizing only for nucleolar transcription, and activity of extranucleolar chromatin is inhibited by both doses.

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MULTIVARIATE DATA ANALYSIS TO STUDY THE EFFECT OF SPACE FLIGHT FACTORS ON NEURONAL STRUCTURE IN THE RAT BRAIN

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KEY WORDS: factor analysis; plastic changes; dendrites

The study of the morphological basis of compensatory processes taking place in the nervous system during adaptation to weightlessness, an important role in the development of which is played by the visual system, has necessitated the study of the state of the visual cortical neurons in the brain of rats exposed to the influence of space flight factors (SFF). Considering the complexity and diversity of interneuronal interactions in the neocortex, it seems problematical that all neurons (even of the same class) would give an identical morphological response to SFF. It is perfectly possible that a quite sizeable group of neurons does not participate in the formation of compensatory processes and does not undergo morphological changes. Consequently the problem arises of how to distinguish from the whole population of experimental neurons, groups of cells that possess a characteristic set of features which is not found in control neurons. The parametric and nonparametric statistical methods (the Kruskal–Wallis test, anovar, the t test) used traditionally [3, 5] in studies of plastic changes in dendrites, utilize a priori information on the subdivision of the test population into groups and cannot help to solve the present problem.

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