EFFECT OF DIFFERENT CONDITIONS OF HYPERBARIC OXYGENATION ON MORPHOLOGY AND TRANSCRIPTION OF CORTICAL NEURONS OF RATS WITH EXPERIMENTAL STROKE

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The protective action of hyperbaric oxygenation (HBO) in stroke has been described by many research workers [3, 7, 8], but its clinical use in the treatment of acute cerebral ischemia still rests on an inadequate basis. The aim of this investigation was to study the trend of the morphological changes and transcription activity of pyramidal neurons in the frontal cortex of rats with acute ischemia, depending on the conditions of HBO.

METHODS

Experiments were carried out on 120 noninbred albino rats weighing 200 g. Two models of ischemia were used: moderately severe — after right-sided ligation (group I), and severe, with bilateral ligation of the common carotid arteries (group II) [1, 2, 6]. The operation was performed under pentobarbital anesthesia. The state of the animals in group I immediately after the operation was satisfactory, and their behavior and general appearance were not visibly different from those of intact animals, yet a few hours later these rats were found to have mild mono- and hemipareses. The animals of group II were in a severe state after the operation. With the passage of time the state of the animals worsened and their mortality increased, so that after 24 h it was 100%. The effect of a single application of HBO on the morphologic and functional state of the frontal cortical neurons of the rats was investigated on these models, when three different conditions of HBO were used: exposure for 1 h to 2.0 atm (absolute atmospheres) with oxygen, and in group II, the same but with air, and exposure for 30 min to 1.2 atm with oxygen, which is the situation closest to that observed clinically [3]. The sessions were given 2 h after the operation. The use of HBO, whatever the conditions, was accompanied after 10-15 min by appreciable improvement of the animals' state. In the animals of group II the cyanosis disappeared, behavior and external appearance returned to normal, and their general state remained good for several hours after HBO. The survival rate of the rats after the operation in this group, following the use of HBO toward the end of the 1st day, amounted to 50% after exposure to 1.2 atm and 30% after exposure to 2.0 atm with oxygen. The animals' state 24 h after HBO at 1.2 atm was rather better than after exposure to 2.0 atm. Structural changes and transcription activity of the large and medium-sized pyramidal neurons in the frontal cortex of the ligated rats was assessed 15 min after a session of HBO and 24 h later. The material was taken from 3-5 animals (except in group II after 24 h, when it was only from 2-3 survivors). Intact rats, rats undergoing a mock operation, and rats undergoing ligation but not treated with HBO, and killed 2.5 h after the operation, served as the control. The number of large and medium-sized preserved pyramidal neurons was counted in several fields of vision and the results averaged.

To determine transcription (template) activity of the chromatin of the neurons studied, the histoautoradiographic method of detecting activity of endogenous RNA-polymerases in fixed neurons in sections was used [10]. The RNA-

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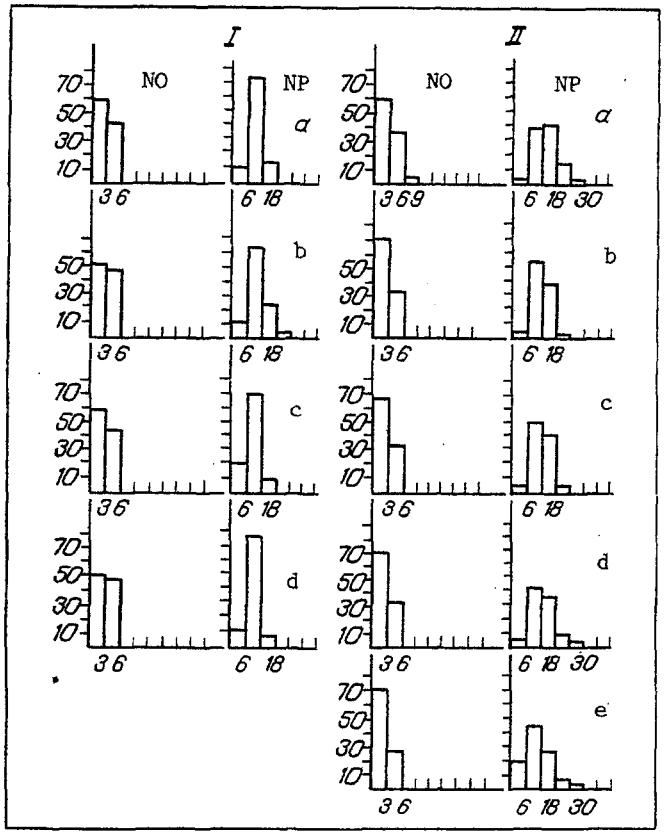

Fig. 1

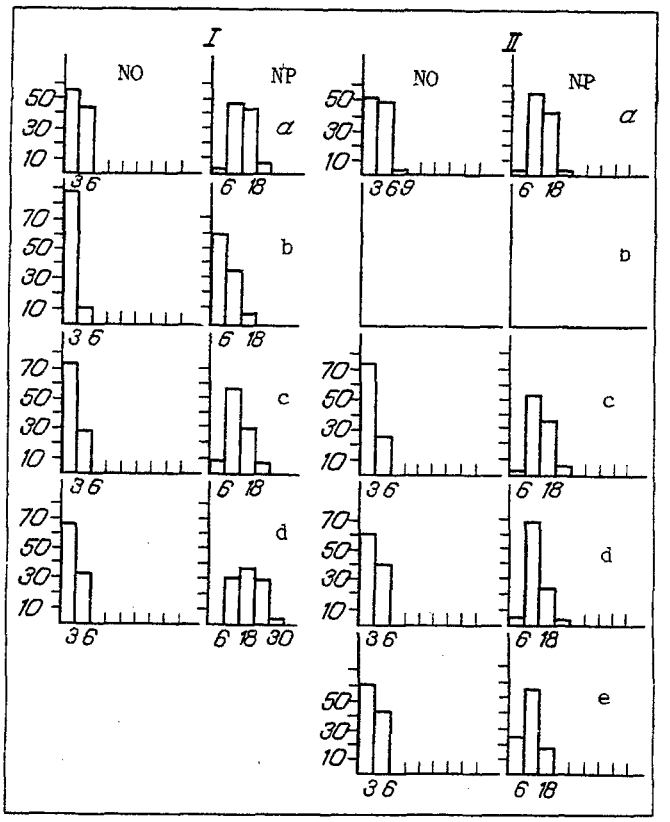

Fig. 2

Fig. 1. Distribution of large and medium-sized pyramidal neurons in frontal cortex of a rat 2.5 h after ligation of common carotid arteries, depending on level of labeling. I) Unilateral ligation; NO) nucleolus; NP) nucleoplasm; II) bilateral ligation: a) control; b) operation without HBO; c) HBO—O_2 at 1.2 atm; d) HBO—O_2 at 2 atm; e) HBO—air, e atm. Abscissa, level of labeling, in conventional units; ordinate, number of cells in % of total number counted.

Fig. 2. Distribution of large and medium-sized pyramidal neurons from rat frontal cortex 1 day after ligation of common carotid arteries, depending on labeling level. Legend as to Fig. 1.

polymerase reaction was conducted by the labeling method. The level of template activity of the chromatin was estimated by counting the number of grains of reduced silver separately above the nucleolus and nucleoplasm. At each experimental point 150 cells were analyzed. The significance of differences between the groups was estimated by Wilcoxon's test and the φ-test.

RESULTS

Some decrease in the average number of preserved neurons in a field of vision (by 20-25% compared with the control) was observed 2.5 h after application of the ligature to the right common carotid artery of animals not receiving HBO. Many of them demonstrated one or other form of chromatolysis. Approximately one-third consisted of elements which had lost their characteristic shape, and were round, with a wrinkled or triangular nucleus and a hypertrophied nucleolus. Vacuolated and shrunken neurons, but only solitary normochromic neurons were seen.

Morphological changes in the frontal cortex had progressed 24 h after the operation. Many neurons (up to 70%) had died by this time. The relatively preserved part of the population consisted of cells with a shrunken nucleus, and with neurons in a state of total chromatolysis.

In the 2nd group, by 2.5 h after the operation the number of preserved neurons per field of vision was about 50%, and the majority of them were vacuolated neurons and cells with total chromatolysis. The morphological changes increased progressively thereafter. The animals' state worsened and death from respiratory arrest took place.

The mean transcription activity of the residual pyramidal neurons 2.5 h after the operation in the animals of group I did not differ significantly from the control, either for nucleolar or for extranucleolar chromatin (Fig. 1), although some increase in the fraction of strongly labeled cells could be seen on the histograms in the case of animals with stroke. However, after 24 h the template activity of those neurons which remained intact was significantly below normal, due to predominance of very weakly labeled cells (Fig. 2).

In the rats of group II a significant decrease in template activity of the extranucleolar chromatin of the preserved neurons was observed as early as 2.5 h after the operation (Fig. 1).

The use of HBO in group I, irrespective of dose, had a normalizing effect immediately after the session on transcription, abolishing the slight activation which took place after the operation (Fig. 1c, d). In group II the effect, which consisted on the contrary of an increase in template activity up to the control level, was obtained immediately after the session only when a dose of 2 atm was given with oxygen (Fig. 1d). This same dose with air, on the contrary, suppressed transcription even more (Fig. 1e).

In group I, 24 h after administration of HBO, a marked protective effect was observed, with preservation of about 70% of neurons in the case of 2. atm and 55% in the case of 1.2 atm, close to the picture at the 2.5 h point without HBO. The neuron subpopulations studied for both doses consisted mainly of neurons with a shrunken nucleus, and also of cells in a state of hydropic degeneration and total chromatolysis. The template activity of the neurons in the case of 1.2 atm was significantly higher and without HBO (Fig. 2, Ic), but after 2.0 atm the fraction of cells with strongly labeled extranucleolar chromatin was actually higher than in the control (Fig. 2, Id).

In group II, 24 h after HBO, a dose of 1.2 atm demonstrated a better protective effect than a dose of 2.0 atm. The number of residual large and medium-sized pyramidal cells was 50-60%; some of them were close to normal, although they often had stained nuclei, and there were few hyperchromic and shrunken cells. In the case of 2.0 atm with oxygen the number of neurons remaining intact by this time was only 25-30%, and most of them, moreover, were in a state of total chromatolysis, sometimes in a severe form. The transcription activity of the neurons remaining intact in this group after exposure to 1.2 atm, for extranucleolar chromatin was close to the control value (Fig. 2, IIc), whereas in the case of 2.0 atm with oxygen it remained depressed (Fig. 2, IId). When 2.0 atm with air was used the number of pyramidal cells remaining intact was of the order of 50%, and many of them were in a state of total chromatolysis, although some normochromic neurons were seen. Meanwhile, transcription activity for this group was lower than in the case of 2 atm with oxygen (Fig. 2, IIe).

The clinical manifestation of bilateral division of the common carotid arteries in these experiments was thus evidently determined by a combination of severe chronic cerebral ischemia and pentobarbital anesthesia, inducing a combination of respiratory disturbances in the animals, intensifying their cerebral hypoxia [4, 6, 9, 11]. Ischemia of the frontal cortex also was accompanied by changes in template activity of medium-sized and large pyramidal neurons remaining intact.

The use of HBO 2 h after ligation of the common carotid arteries had on the whole a marked protective action, delaying the development of destructive processes in the frontal cortex and preventing depression of transcription. Other workers also have noted a stimulating effect of HBO on transcription [5]. Dissociation of the aftereffect of different conditions of HBO in severe ischemia was discovered for the first time in our experiments: 1 day after application, HBO in a dose of 1.2 atm demonstrated a stronger protective effect with respect to preservation of pyramidal neurons than a dose of 2 atm.

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