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AGE FUNCTIONAL PECULIARITIES OF THE GINGIVAL TISSUE RESPONSE IN RATS TO DISCONTINUOUS HYPOBARIC HYPOXIA AND PHOTOPERIOD OF A DIFFERENT DURATION

Abstract. *The experiments conducted on albino rats of different ages and gender have found that systemic incontinuous long-term hypobaric hypoxia causes inconsiderable reduction of intensity of lipid peroxide oxidation and proteins in the gingival tissues, and considerable reduction of enzymatic activity in them. Antioxidant-prooxidant index is indicative of the advantage of the antioxidant activity in the gingival tissues according to the intensification of resistance processes of these tissues.*
Key words: *gums, hypoxia, photoperiod, oxidized-modified proteins, gender.*

Introduction. Any stressful situation including hypoxia within the norm is associated with generation of oxygen active forms (OAF) [1], participating in the processes of activation of the transcription factors and appropriate genes including those coding antioxidant enzymes [2, 3]. Protein molecules are targets for the action of OAF resulting from the formation of oxidized products of radical and non-radical nature. Destruction of such oxidized proteins is considered as a sign of antioxidant protection [4]. Moreover, contrary to lipids nucleic acids are considered to be oxidized first. First of all OAF is oxidized and therefore oxidation-modified proteins are suggested to be one of the earliest and most accurate markers of tissue damage [5]. Our previous studies detected gender peculiarities of the response of lipid peroxide oxidation (LPO) molecular products and oxidation-modified proteins (OMP) of the gingival tissues in immature rats to the photoperiod of a different duration [6, 7].

Objective: to detect age peculiarities of response of the pro- and antioxidant, proteolytic and fibrinolytic systems of the gingival tissue and blood plasma in rats under conditions of a combined action of the systemic incontinuous hypobaric hypoxia and the photoperiod of a different duration.

Materials and methods. The study was conducted on 28 albino nonlinear mature male rats and mature and immature female rats. The examinations were performed according to the main regulations of GLP (1981 p.), Requirements of work with the use of experimental animals (1977 p.), the Council of Europe Convention on protection of vertebrate animals used in experiments and other scientific purposes dated 18.03.1986, the Directive of EEC №609 dated 24.11.1986 and the Order of the Ministry of Public Health of Ukraine №281 dated 01.11.2000.

To conduct experimental studies an original method was designed with simultaneous application of a long-term incontinuous hypoxic effect with photoperiod changes of different directions. Hypobaric hypoxia was simulated, equivalent to the height of 4 000 m above sea level created in a transparent flowing hermetic camera by means of air suction with the help of a vacuum compressor. The speed of "rise" of animals to this value was 24 km/hour. Hypoxia serials 2 hour long were conducted every day from 9 to 11 a.m. during 14 days against the ground of three light regimens: natural light peculiar for spring-summer period with duration of day light of 15 hours; continuous 24-hour light with the intensity of 500 lux and complete darkness during 24 hours. A changed regimen of light was introduced since the first series of hypoxia, and the animals were under changed photoperiod during 15 days.

On the following day after the last series of hypoxia the animals were removed from the experiment by means of decapitation under ether narcosis. Euthanasia was performed in the morning from 9 to 12 a.m. for all the groups of animals. After exsanguination the samples of the gingival tissue were quickly taken, weight on the torsiobalance and homogenized in 1,2 ml of cool TPIC-buffer and 2 ml of cool borate buffer. The homogenate was frozen and kept in the freezer at the temperature of – 20˚C before performing laboratory examinations.

Results and discussion. In the intact animals the processes of free radical lipid oxidation and accumulation of molecular LPO products manifested more in mature male rats as compared to immature male rats: diene conjugate and Malone aldehyde

became in 1,5 and 1,3 times higher. Differences in the antioxidant system of the gums were the following: catalase activity in the immature rats was 16,64% higher than that of the mature ones. At the same time both in immature and mature rats superoxide dismutase (SOD)/catalase balance was practically the same. Hence, antioxidant-prooxidant index (API)/peroxide oxidation in the gingival tissues of immature male rats was in 1,6 times higher than that of mature ones. It is indicative of a well-developed system of the antioxidant protection, that is, before the production of testosterone by the testes.

Interval hypobaric hypoxia (4000 m above the sea level) produced more intensive effect on the immature male rats as compared to the mature male rats. In immature male rats as compared to the mature male rats, interval hypoxia reduced the processes of free radical lipid oxidation and accumulation of LPO molecular products (diene conjugate and Malone aldehyde became in 1,6 and 1,4 times lower) and SOD activity - in 1,3 times. In mature male rats the integral index (API) did not undergo considerable changes and was higher than that of the intact mature male rats and those suffered from interval hypoxia, which can be considered as a high level of the antioxidant system tension.

Staying of animals during 15 days under conditions of a continuous light ("physiological" epiphysectomy) intensified the processes of free radical lipid oxidation and more considerable accumulation of LPO products in the gingival tissues as compared to immature animals. At the same time, AO/PO index in the mature rats was 1/3 more than that of immature male rats. Interval hypoxia against the ground of light intensified even more the age difference in the response of the gingival tissues.

The results of a series of experiments with continuous light and hypoxia are indicative of the fact that in mature male rats adaptive abilities are more developed that those in the immature ones.

The experiments with melatonin-forming function of the epiphysis demonstrated that a long-term keeping of rats in darkness resulted in an increased accumulation of LPO products in the gingival tissues of the immature animals, reduced the activity of antioxidant enzymes and SOD/catalase balance as compared to the mature animals. The integral index of correlations between the antioxidant activity and the content of LPO products (AO/PO) was identical both in immature and mature male rats. Although, in immature animals the condition was maintained by means of increasing enzymatic activity in response to a high level of LPO products. Keeping mature male rats in darkness reduced the level of LPO products and activity of SOD and catalase in the gingival tissues. We suggest that, first of all, it was the result of anti-stress and antioxidant effect of melatonin. Interval hypoxia in the period of keeping male rats in darkness affected the gingival tissues of both age groups approximately in a similar way as the effect of hypoxia alone. Comparing the results of changes of pro- and antioxidant processes in the gingival tissues in the three series of the experiment a positive protective effect of keeping the animals in darkness is seen, that is melatonin effect.

In intact immature animals LPO products and enzymatic activity were found to be less and higher respectively than those in mature rats. The interval hypoxia (14 days) does not affect much on the pro- and antioxidant processes in the gums of mature rats, although it reduced their levels in immature rats. A long-term light "physiological epiphysectomy" in mature rats intensifies pro- and antioxidant processes in the gums, and immature male rats it does not affect the content of LPO products and reduces activity of antioxidant enzymes. The interval hypoxia against the ground of a long-term light in mature rats increases the integral index of correlation between antioxidant activity and the content of LPO products in the gingival tissues, and in immature rats it decreases the value of this index. A long-term keeping in darkness (stimulation of melatonin production by the epiphysis) in mature animals reduces and in immature animals increases the level of LPO products in the gums and activity of antioxidant enzymes.

The comparative analysis of proteolytic processes in the gingival tissues under the action of a long-term hypobaric incontinuous hypoxia (2 hours a day) and changed photoperiod during 15 days is presented in

Under the action of incontinuous hypoxia the values of proteolytic activity of all the three types of proteins in immature male rats were less than those in mature male rats. It was especially manifested for collagen – 64,25%, high molecular proteins (35,82%) and less – for low molecular prteins – 19,83%).

The change of photoperiod duration, especially continuous darkness found a number of age peculiarities. Thus, under the action of light only low molecular lysis in the gums of immature male rats was 12,69% less than that of mature male rats, other indices of lysis were similar in both age groups. Under conditions of darkness a considerable intensification of proteolytic activity in immature male rats was found as compared to mature ones.

Certain attention is drawn to the fact that collagenolysis was the most pronounced sign of proteolysis age differences in the gums. Thus, under conditions of continuous light alone and under conditions of its action together with hypoxia collagenolysis was similar in mature and immature

male rats. At the same time, under conditions of darkness and its combination with hypoxia collagenolysis was stimulated much more 51,82% up in immature male rats as compared to mature ones.

Conclusions. There are age peculiarities in physiological system of gum protection. In mature male rats under conditions of continuous light the balance of superoxide dismutase and catalase is preserved, and the integral index of the gum protective system remains unchanged together with increasing level of oxidized-modified proteins and reduced proteolytic processes. In immature rats the balance of antioxidant enzymes is disturbed, and the index of the gum protective system is reduced together with a reduced content of oxidized-modified proteins and active proteolysis.

Prospects of further studies. Further comparative studies of proteolysis processes in the gingival tissues of mature and immature male rats can give additional information concerning the suggested differentiation of factors of the gingival tissue gender peculiarities.

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