CERULOPLASMIN–TRANSFERRIN ANTIOXIDANT SYSTEM OF RATS DURING HYPERBARIC OXYGENATION

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Hyperbaric oxygenation (HBO) is used in the treatment of several diseases [2, 7]. Meanwhile the use of HBO has some limitations because of the toxic action of oxygen on the patient, which may be due to activation of lipid peroxidation (LPO) in the tissues and, in particular, in the blood serum [2, 6]. For this reason the study of the mechanisms of regulation of LPO processes in the blood serum during HBO is of great interest.

In the investigation described below changes in activity of the ceruloplasmin–transferrin antioxidant system (AOS) and in the serum level of LPO products were studied in rats subjected to HBO.

EXPERIMENTAL METHOD

Experiments were carried out on 130 Wistar rats weighing 120-140 g, kept on the usual animal house diet. Three animals were put in a 13-liter pressure chamber. The duration of "ascent" was 5 min and of "descent" 7 min; the exposure to a given pressure was 25 min. Control animals were kept in the chamber in air at atmospheric pressure for the same length of time. The excess of carbon dioxide was removed by blowing oxygen continuously through the chamber. The experimental animals were decapitated 10 min and 2 and 24 h after the 1st, 3rd, 5th, 7th, and 9th sessions of HBO, and control animals were decapitated 10 min after the procedure. The malonic dialdehyde (MDA) concentration in the blood serum was determined by the method in [9]. For this purpose 0.5 ml of blood serum and 1 ml of a 0.5% aqueous solution of thiobarbituric acid (TBA) were added to 3 ml of 1% phosphoric acid. The mixture was incubated for 40 min at 100°C, after which the MDA–TBA complex was extracted with normal butanol. The MDA concentration was judged from the intensity of absorption of the butanol fraction at 532 nm, using two base points, namely 515 and 550 nm. The coefficient of molar extinction was taken to be 1.5×10^5 M^{-1} cm^{-1} [9].

To record EPR spectra 0.2 ml of serum was introduced into a cylindrical PVC mold, frozen in liquid nitrogen, and then expressed from the mold. The specimens were transferred into a Dewar flask with a projecting finger, which was placed in the resonator of a "Varian E-4" EPR spectrometer. EPR spectra were recorded under the following conditions: frequency of the klystron oscillator 9.03×10^9 Hz, amplitude of modulation 6.3 G; superhigh-frequency power 10 mW. The amplitude of the EPR signal of ceruloplasmin (CP) with g = 2.05 and the amplitude of the EPR signal of transferrin (TR) with g = 4.3 were measured on the spectrum. Antioxidative activity (AOA) was estimated as the ratio of the EPR CP/TR signals [5]. The results were analyzed by EMG-666 microcomputer, by calculating the mean value of the parameter and its standard error.

EXPERIMENTAL RESULTS

The conditions of HBO used in the investigation are considered to be toxic for man. Meanwhile preliminary investigations showed that external features of oxygen poisoning (convulsions) appeared in only 1.5% of rats after 5-7 sessions. This indicates that these conditions of HBO are not toxic for rats, in agreement with the already known fact that these

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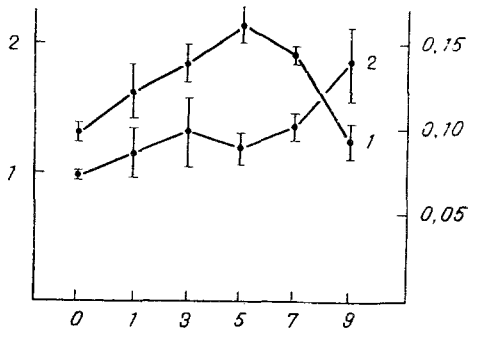

Fig. 1. Changes in parameters of rat blood serum (MDA and CP/TR) depending on number of HBO sessions. Abscissa, number of HBO sessions; ordinate: left — CP/TR (in relative units), right — MDA level (in μM), 1) MDA, 2) CP/TR.

TABLE 1. Changes in Parameters of Rat Blood Serum (MDA, CP, and CP/TR) at Intervals after the 5th HBO Session (M ± m)

Parameter	Control	Time of taking blood after 5th session		
		10 min.	2 h	24 h
MDA, μM	0,100±0,009	0,160±0,013	0,144±0,014	0,110±0,016
CP, relative units	18±5	19±1	22±4	36±7
CP/TR, relative units	1+0,04	1,05±0,09	1,20±0,20	1,78±0,33

animals are resistant to the action of oxygen [3]. The so-called "aftereffect" of hyperbaric oxygen [1] is that during HBO several of the parameters of the animal or person treated undergo significant changes after the HBO session has ended. To allow for such effects, the serum of the experimental animals was studied immediately after the session and during the next 24 h. It was shown that the MDA concentration rose steadily until the 5th session of HBO (Fig. 1). The increase in MDA concentration in the blood serum immediately after each session could be connected with activation of LPO in response to an increased partial pressure of oxygen [2, 6]. The fact that the serum MDA level rose with an increase in the number of sessions may be explained by a change in the lipid composition of the blood. For instance, it was shown previously [4] that under HBO conditions blood levels of total lipids, fatty acids, and cholesterol all rise. In addition, a change in the velocity of LPO processes may be associated with a change in activity of the antioxidative systems of the blood serum.

After the 5th session the MDA concentration began to fall, and after the 9th session the serum MDA level of the experimental animals was the same as that of intact rats.

It can be tentatively suggested that the fall of the LPO level in this case was connected with activation of certain AOS in the rat serum, for example, the CP/TR system.

It follows from these results that during the first five sessions the ratio between EPR signals CP/TR was unchanged (Fig. 1). After the 5th session the CP/TR ratio began to increase, until the 9th session, reflecting an increase in serum AOA [5]. Thus the MDA concentration in the blood serum during HBO rises until the CP/TR AOS is activated. It can be postulated that the subsequent fall in the serum MDA concentration was the result of activation of this system.

Measurement of the MDA concentration over a period of time, i.e., immediately after the session and again 2 and 24 h later, demonstrated in all cases a tendency for this parameter to fall. However, a significant fall of the MDA level was observed only after the 3rd and 5th sessions. The MDA concentration 24 h after the session did not differ from that in the blood serum of the control animals.

The CP/TR ratio changed significantly only during the 24 h after the 5th session. Unlike MDA, the CP/TR ratio under these circumstances did not fall, but rose during this time interval (Table 1).

After the 5th session changes in activity of the CP/TR AOS were out of phase with changes in the MDA concentration (Table 1), which suggests that these processes are interconnected: a

rise of the serum AOA leads to a fall of the LPO level. This view is supported also by the strong negative correlation between the CP/TR ratio, reflecting the serum AOA, and the quantity of MDA accumulating in it ($r = -0.72$). The results of this investigation and also those obtained by other workers [8, 10] are evidence of the important role of the CP/TR system in the regulation of serum LPO processes. On this basis the following chain of events taking place during HPO can be postulated. An increase in the partial pressure of oxygen in the blood serum leads to activation of LPO in it [2]. This process is accompanied by a change in the lipid composition of the blood serum [4, 5], which leads to further accumulation of MDA after each successive HBO session. Later, after the 5th session, activation of the serum AOS takes place. One possible cause of the activation of this system may be a high level of LPO products in the serum. Later, against the background of high AOA, LPO processes are not activated during the HBO session. It can thus be postulated that the CP/TR system participates in protection of the body against the toxic action of oxygen.

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EFFECT OF NALOXONE ON IMMOBILIZATION-INDUCED HYPOALGESIA IN RATS

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Neurochemical investigations have demonstrated the involvement of both opioidergic and nonopioid mechanisms in the production of stress-induced hypoalgesia, and the prevalence of one or the other depends on the nature and duration of the stressor factors and also on activity of the animal based on them [1, 4, 6, 10]. A study of hypoalgesia developing in rats during and after short-term immobilization showed that blocking opiate receptors with naloxone does not reverse changes in the latent periods (LP) of tail withdrawal in the tail-flick test [14] or of licking the paws in the hot-plate test, although it weakens changes in nociception assessed on the basis of jumping by the animals [4]. However, there are no data on the dynamics of the change in pattern of nociceptive sensation during long-term immobilization stress and on the involvement of endogenous opioidergic mechanisms in its production.

The aim of this investigation was to study LP of nociceptive responses of rats during and after immobilization for 24 h, and also to determine the effect of naloxone, a specific blocker of opiate receptors, on these periods.

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