EFFECT OF EXTREMAL FACTORS AND THE ANTIOXIDANT α-TOCOPHEROL ON INTENSITY OF CHEMILUMINESCENCE OF BLOOD PLASMA

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Activation of free-radical oxidation is a common stage in the development of many pathological states [12]. Accordingly, the search for ways of optimizing the conditions for their pharmacologic correction has assumed particular urgency. We know that chemiluminescence provides the most accurate method of determining the concentration of free radicals and of assessing the efficiency of working of the antioxidant protection systems [14]. A general shortcoming of all known modifications of the method is the need to obtain macroquantities of biological material on which to undertake the measurements [6, 7], so that dynamic monitoring of the direction of the change in the parameters studied is difficult. For this reason the present writers have developed a sensitive micromethod, based on recording the kinetics of the "fast flash" of Fe^{2+}-induced chemiluminescence (ChL) of blood plasma.

The aim of this investigation was to estimate the informativeness of this method for studying free-radical oxidation processes in the blood plasma of experimental animals.

EXPERIMENTAL METHOD

Experiments were carried out on 220 noninbred male albino rats weighing 200-250 g. Five schedules of oxygen deficiency were simulated. Acute hypobaric hypoxia was created in a pressure chamber by "lifting" the animals to an "altitude" of 9000 m at the rate of 33 m/sec and exposing them for 10 min to those conditions. The resistance of the rats to oxygen deficiency was estimated on a model of acute hypobaric hypoxia (AHBH) [2], using the survival rate an "altitude" of 11,000 m as the criterion. Moderate normobaric hypoxia was simulated by keeping the animals for 2 h in a medium containing 10% oxygen and 90% nitrogen (NHM-10). Partial cerebral ischemia was created under anesthesia by ligating the common carotid arteries for 3 h. Inhalation of smoke from "Stolichnaya" cigarets (from the "Yava" factory) by the rats was carried out in vacuum chambers, with a ratio of air: smoke of 1:4 for 3 months, 5 days a week (two cigarets each day), and with intervals of 2 days. Samples for chemiluminescence analysis to study correlation between the resistance of the animals to acute oxygen insufficiency and the intensity of the "fast flash" of ChL were taken once only — before the rats were tested in the pressure chamber, whereas to study the action of AHBH they were taken twice: before and immediately after the end of exposure to this factor; to investigate the effect of moderate hypobaric hypoxia, of cerebral ischemia, and of tobacco smoke, samples were taken at frequent intervals in the course of the experiments. As antioxidant, the animals were given an intramuscular injection of an oily solution of α-tocopherol acetate in doses of 10, 50, and 100 mg/kg. Animals of the control group received the oily base of the preparation. Its action was assessed after 24 h. The kinetics of ChL was recorded on a special apparatus, the design of which was described previously [10]. To carry out the experiment 3.0 ml of phosphate buffer (20 mM KH_2PO_4, 105 mM KCl, pH 7.4), 1.0 ml of eosin (2.7 mM), and 4 μl of blood plasma were introduced into

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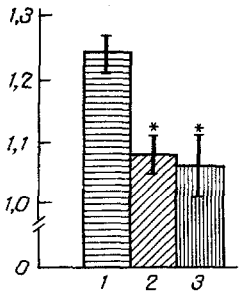

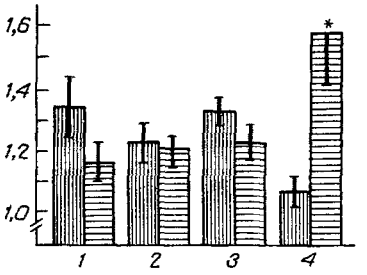

Fig. 1 Fig. 2

Fig. 1. Value of parameter Iff in groups of animals differing in resistance to hypoxia. Groups of animals with low (1), average (2), and high (3) resistance to hypoxia. Ordinate, Iff — intensity of "fast flash" of ChL (in relative units). Asterisk indicates statistically significant difference ($p < 0.05$) between the given group and group of animals with low resistance to hypoxia.

Fig. 2. Parameter Iff during chronic inhalation of tobacco smoke by rats. Vertically shaded columns — animals of control group; horizontally shaded — experimental group. Abscissa, time after beginning of exposure: 1) 2 weeks, 2) 1 month, 3) 2 months, 4) 3 months; ordinate, Iff — intensity of "fast flash" of ChL (in relative units). Asterisk indicates statistically significant difference ($p < 0.05$) between experiment and control.

a measuring cell. After incubation for 2 min with continuous mixing, 1.0 ml of $FeSO_4 \cdot 7H_2O$ (38.5 mM) was added to the system and fluorescence was recorded for 20-30 sec. The intensity of the "fast flash" of ChL (Iff, in relative units) was determined. Fluorescence of the control sample, not containing blood plasma, was then recorded. The end results were shown in the form of indices, namely ratios between the corresponding parameters:

$$Iff = Iff_{(exp)}/Iff_{(c)}.$$

EXPERIMENTAL RESULTS

Recording ChL of the blood plasma of intact rats revealed considerable variation of individual values of the parameter Iff (between 0.92 and 1.55), evidently as a result of differences in the overall efficiency of working of the tissue antioxidative protection systems of the individuals tested. The data in the literature showing correlation between activity of enzyme systems regulating the intensity of free-radical reactions and the resistance of the animal to oxygen deficiency [13] provided a basis for undertaking appropriate studies of this parameter. The results of testing animals on the AHBH model were used to divide the total population into three groups: those with low resistance to hypoxia (LR, n = 28, mean survival time at an altitude" of 11,000 m, T = 1.48 ± 0.15 min), average (AR, n = 18, T = 5.27 ± 0.36 min), and high (HR, n = 8, T = 13.20 ± 1.48 min). The animals constituting the LR group were found to have a higher initial value of Iff than animals of the other two groups (Fig. 1), but no significant difference was found between the value of this parameter in the latter. The use of this arbitrary criterion enabled a distinction to be drawn between animals with low resistance and the rest of the population with a 90-95% level of accuracy. The reliability of determination of individuals with increased resistance to oxygen deficiency was somewhat lower (the error of prediction, compared with the results of standard testing, was 15-20%).

To assess the informativeness of the method, it was interesting to compare the effect of exposure to extremal factors of different kinds on the value of the parameter Iff.

TABLE 1. Intensity of "Fast Flash" of ChL during Exposure to Acute Hypobaric Hypoxia and to Cerebral Ischemia

Factor	Duration of exposure, min	Iff, % of initial level
Acute hypoxia (h = 9000 m)	10	108,1±4,9
Cerebral ischemia	5	98,3±4,4
	15	94,1±7,3
	30	99,8±6,9
	60	99,1±4,2
	180	121,4±14,4

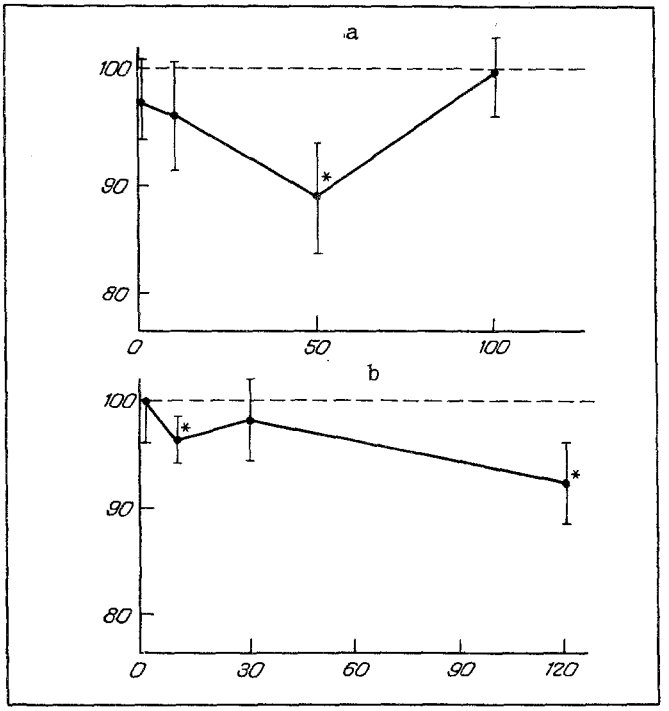

Fig. 3. Parameter Iff following injection of α-tocopherol into animals (a) and exposure to normobaric hypoxia (b). Abscissa: a) dose of compound (in mg/kg); b) duration of exposure to normobaric hypoxia (in min); ordinate, Iff, intensity of "fast flash" of ChL (in % of initial level). Asterisk indicates statistically significant difference ($p < 0.05$) compared with initial level of Iff.

It was found that when the animals were exposed to acute hypobaric hypoxia (at an "altitude" of 9000 m) and when cerebral ischemia was created, the average amplitude of the "fast flash" of ChL remained unchanged throughout the experiment (Table 1), even though toward its end, those animals who were least resistant to the action of these extremal factors were in a preagonal or agonal state, and according to some investigators [3, 5], this state must correspond to marked activation of lipid peroxidation (LPO). Meanwhile, in chronic inhalation of tobacco smoke by the rats (a factor of more moderate intensity) the parameter Iff rose statistically significantly toward the end of the 3rd month (Fig. 2).

Comparison of the results with interpretation of the mechanisms leading to the development of the "fast flash" of Fe^{2+}-induced ChL [11] suggests the possible causes of the apparent paradoxical nature of the results. In the modification of the method proposed above the main contribution to the formation of the "fast flash" of ChL is made by free-radical processes taking place in the aqueous phase and, in part, connected with generation of active forms of oxygen (AFO). We

know that enzyme complexes involved in protection from AFO in the tissues of the intact organism work with an adequate reserve of stability [9]. Nevertheless, during exposure to AHBH and the development of a state of cerebral ischemia, the power of these systems is insufficient to prevent activation of free-radical oxidative processes, and more especially, the intensification of LPO. The compensatory reaction of an increase in activity of enzymes acting as quenchers of AFO [4] is not yet capable of effectively protecting the tissues and organs against the action of damaging factors, but can maintain an unchanged, or only slightly increased, intensity of the "fast flash" of ChL. Meanwhile, long-term exposure to damaging factors of moderate intensity can induce intensification of free-radical processes as a result of gradual inactivation of enzymes concerned in antioxidative protection by intermediates and by end-products of LPO and through exhaustion of the pool of quenchers of AFO, a situation which may be accompanied by an increase in the value of Iff.

Injection of the antioxidant α-tocopherol into the animals in a dose of 50 mg/kg led to a statistically significant decrease in the value of Iff (on average by 11.2%) (Fig. 3a). In doses of 10 and 100 mg/kg α-tocopherol affected the amplitude of the "fast flash" of ChL: whereas in the first case its concentration was too low to exhibit antioxidant properties, in the second case it was evidently too high, and led to the development of the pro-oxidant effect of α-tocopherol which has been described in the literature [1]. Short-term adaptation of animals to normobaric hypoxia (NHM-10) led to a similar but less marked change in the parameter Iff (Fig. 3b), possibly because of the stimulating action of moderate oxygen deficiency on the antioxidant systems of the body [8].

It can be concluded from analysis of the results that the micromethod we have developed is highly informative from the standpoint of predicting the resistance of the individual to hypoxia, and also when determining the efficacy of pharmacotherapy and also of nonpharmacological procedures activating the endogenous antioxidative protection system.

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