FREE-RADICAL OXIDATION AND ANTIRADICAL DEFENSE OF THE BRAIN DURING ADAPTATION TO CHRONIC STRESS

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A key role in the activation of free-radical lipid peroxidation (FRLPO) in the damaging effects of stress has been convincingly demonstrated in recent years [1, 5, 7]. Despite an abundance of data on prevention of activation of FRLPO by antioxidants during stress [4, 5], the mechanisms of adaptation to activation of FRLPO have so far received little study. According to Selye [8], the response of the body to stress is realized as the general adaptation syndrome, which incorporates phases of alarm, resistance, and exhaustion. Further elaboration of the adaptation theory led to the discovery of four basic stages [6]: emergency adaptation, the transition from emergency to long-term adaptation, long-term adaptation, and exhaustion. The stage of emergency adaptation is characterized by the presence of a marked stress reaction and activation of FRLPO processes; activation of FRLPO also is observed in the exhaustion stage [6].

The aim of this investigation was to study parameters of FRLPO and of the antiradical defense of the brain during the development of long-term adaptation to chronic emotional-painful stress in rats. It was interesting to compare the results with the corresponding parameters of the blood serum (to discover any possible specificity of the brain response) and with changes in some physiological parameters during adaptation.

EXPERIMENTAL METHOD

Experiments were carried out on 20 noninbred male albino rats weighing 190-220 g. A combination of electrodermal stimulation in the version of the "expectation stress" model and of the asthenizing action of intermittent white noise [1, 4] for 1-3 weeks, was used as the stress factor (preliminary investigations showed that during the 1st week of action of stress the emergency adaptation stage is produced, but after 3 weeks exhaustion and depth of the animals may take place). The vegetative functions of the animals [4] were assessed at rest and during function testing by immobilization, as well as behavior in the open field test. The animals were then decapitated, and state of the internal organs was determined, blood was collected, and the brain removed. Blood levels of FRLPO products reacting with 2-thiobarbituric acid (TBA) [11] and of conjugated dienes [2] and nonenzymic superoxide-scavenging activity [10] were determined. The cerebral cortex was homogenized and superoxide dismutase activity and concentrations of TBA-reactive [11] and primary molecular products of FRLPO in the homogenate were determined. Lipids were extracted [9] from the remaining portion of brain homogenate and the content of phospholipids and cholesterol in the lipid extracts was determined by thin-layer chromatography [12] and their antioxidative activity was determined in the cumene model [3].

EXPERIMENTAL RESULTS

During investigation of the internal organs of rats exposed to stress for 1 week (group 1), 2 weeks (group 2), and 3 weeks (group 3) for the presence of components of "Selye's triad" [8], changes compared with the control were found only in group 1: the relative values of the weight of the thymus, spleen, and adrenals were 18, 21, and 27% lower respectively ($p < 0.01$), and in 23% of cases gastric ulcers were found. The vegetative parameters, namely arterial blood pressure and Hildebrandt's index, showed phasic changes depending on

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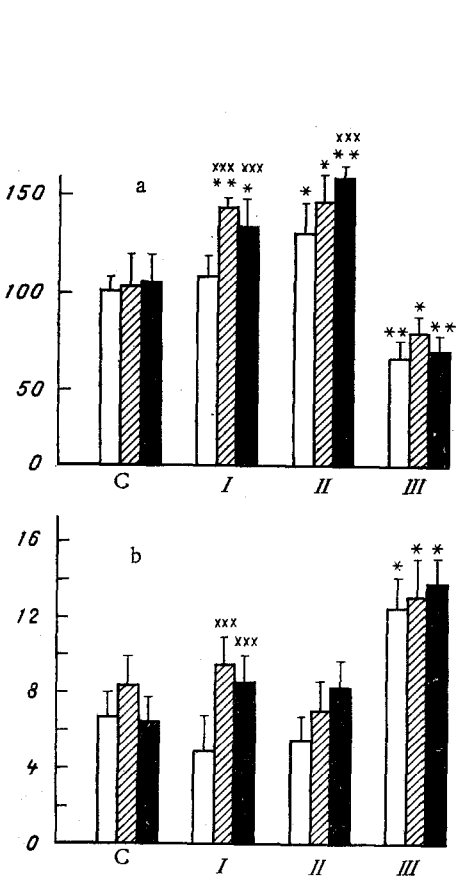

Fig. 1

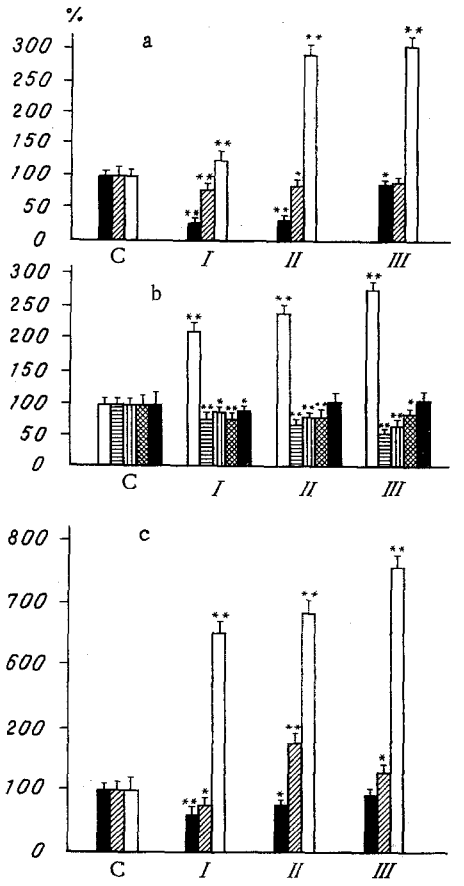

Fig. 2

Fig. 1. Changes in vegetative parameters in chronic emotional-painful stress. Ordinate: a) blood pressure (in mm Hg); b) Hildebrandt's index. Unshaded columns — background, obliquely shaded — after 1 h of immobilization, black columns — after 2 h of immobilization. C) Control; I) 1 week of stress; II) 2 weeks of stress; III) 3 weeks of stress. *p < 0.01, **p < 0.001 compared with control; ***p < 0.01 compared with background within the same group.

Fig. 2. Changes in parameters of FRLPO in brain and blood serum during chronic emotional-painful stress. a) Ordinate, concentration of TBA-active products (black columns); of conjugated dienes (obliquely shaded columns), and superoxide dismutase activity (unshaded columns) in brain homogenate; b) ordinate, concentration of fluorescent products (unshaded columns), cholesterol (horizontal shading), phospholipids (vertical shading, cholesterol:phospholipids ratio (cross-hatching), and antioxidant activity (black columns) in lipid extracts of brain homogenate; c) ordinate, concentration of TBA-active products (black columns), conjugated dienes (oblique shading), and nonenzymic superoxide-scavenging activity (unshaded columns) of blood serum. Corresponding control values taken as 100%. remainder of legend same as Fig. 1.

the time of stress (Fig. 1a, b). In the rats of group 1 pressure was increased in response to immobilization while the background values were unchanged, in the animals of group 2 hypertension was observed, whereas in group 3 the pressure was sharply reduced. Hildebrandt's index, correspondingly, was increased in the rats of group 1 in response to immobilization, and sharply increased in the rats of group 3. The open field test revealed a sixfold increase in the latent period and an increase of 1.6 times in the number of defectations for animals of group 1, and these parameters returned close to the control levels in the rats of groups 2 and 3. The number of squares crossed by the rats of group 1 was 2.2 times less than in the control, but in groups 2 and 3 it was 2.7 and 3.4 times, respectively, greater than in group 1.

The brain of the animals of group 1 has a lower concentration of TBA-active products and conjugated dienes than in the control. With an increase in the length of exposure to stress the concentrations of these FRLPO products in the rats of group 3 increased, the approached the control values (Fig. 2a). The concentration of end products of FRLPO, namely fluorescent Schiff bases, was much higher in the animals of group 1 and continued to rise slowly in groups 2 and 3. Accumulation of fluorescent products against the background of a fall in the concentrations of other FRLPO products can be explained by the extremely slow metabolism of Schiff bases in vivo: the high concentration of them which was observed may have been the result of activation of FRLPO in the initial stage of action of stress. The superoxide dismutase activity of the brain was sharply increased in the rats of group 2 compared with that in the animals of group 1 and the control group. The antioxidant activity of the brain lipids, which was depressed in the rats of group 1, reached normal values in the animals of groups 2 and 3 (Fig. 2b). Concentrations of phospholipids and cholesterol fell progressively with an increase in the duration of stress, but the cholesterol:phospholipids ratio, which was minimal in group 1, subsequently rose slowly (Fig. 2b).

The blood serum of the rats of group 1 characteristically had a low concentration of FRLPO products but high superoxide scavenging activity (Fig. 2c). In the animals of groups 2 and 3 an increase in the content of FRLPO products was observed, with a small subsequent increase in superoxide-scavenging activity.

A combined study of several vegetative, behavioral, and neurobiochemical correlates during adaptation to chronic stress, which was undertaken in the course of the present investigation, enabled three stages of development of long-term adaptation to be characterized. The first stage (group 1) corresponds to the transition from emergency adaptation to long-term adaptation, distinguished by Meerson [6]. Our own data are evidence of strengthening of the adaptive systems (in particular, on account of marked systematic activation of superoxide scavenging) and diminution of the stress reaction (a decrease in the concentration of FRLPO products). A decrease in the chloesterol:phospholipids ratio improves the structure and function of the membranes, thereby permitting effective working of repair and adaptive mechanisms. Labilazation of vegetative parameters, predominance of the fear reaction, and depression of orienting and investigative behavior, involution of the thymus and spleen, and the presence of gastric ulcers in some animals indicate the transitional nature of this period, reflecting the search for optimal levels of functioning of the body during prolonged exposure to stress.

The second stage (group 2) corresponds to the stage of established long-term adaptation [6]. The tactics of the animal's behavior are restored to normal, pressure stabilizes at a higher level, the effectiveness of the antiradical defense of the brain is maximal, FRLPO is maintained at a low level, and the antioxidative activity of the lipids is normalized.

The third stage (group 3) corresponds to a period not described previously, which we define as the beginning of the transition from long-term adaptation to exhaustion: the blood pressure falls sharply and Hildebrandt's index rises, evidence of disturbance of coordination between function of the cardiovascular and respiratory systems, and hyperactivity is observed in the open field test. The concentration of FRLPO products in the brain and blood rises, to reach the control level, and the superoxide-scavenging activity of the brain and blood serum is stabilized. Despite maintenance of the normal level of antioxidative activity of the brain lipids, the cholesterol:phospholipids ratio rises and approaches the control level, whereas the phospholipid concentration falls sharply.

It must be pointed out that the sharp rise in superoxide-scavenging activity of the serum procedes an increase in the brain dismutase activity, and accumulation of conjugated dienes in the serum begins sooner than in the brain, i.e., activation of the antiradical defense of the whole organism is realized faster than in the brain, but during chronic exposure to stress the brain is more resistant to activation of FRLPO than the body as a whole. Attention is also drawn to the fact that during 1 week of stress the accumulation of Schiff bases is maximal at a time of minimal activation of superoxide dismutase in the brain, but the 2nd week of maximal enzyme activity corresponds to minimal accumulation of fluorescent products. This fact is evidence of the key role of scavenging of superoxide radicals in the antiradical defense of the brain.

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RELATIVE EFFECTIVENESS OF METHODS USED TO ISOLATE SPECIFIC RABBIT IgG FOR ELISA DETECTION OF HUMAN PLACENTAL ALKALINE PHOSPHATASE

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Methods of solid-phase enzyme immunoassay (ELISA), which are well-adapted for mass testing and have many advantages over other immunologic methods, have achieved widespread popularity in research and clinical practice [1]. The main components determining the sensitivity and specificity of immunoenzyme (IE) test systems are polyclonal or monoclonal antibodies. Sensitive and specific tests have been created on the basis of monoclonal antibodies [9], but their use is not indicated in every case. When polyclonal antibodies from sera of hyperimmunized animals are used the characteristics of the test systems largely depend on the method of obtaining specific antibodies from the antisera [2, 4]. Methods of ion-exchange and affinity chromatography are used most frequently for these purposes [3, 6].

The aim of this investigation was to compare the effectiveness of isolation of specific rabbit IgG antibodies from antisera by different chromatographic methods for ELISA determination of the quantity and functional activity of human placental alkaline phosphatase (HPAP). HPAP is a biochemical marker of neoplastic cell growth, and determination of its serum level is an important indicator in certain neoplastic diseases [7].

EXPERIMENTAL METHOD

To obtain antiserum rabbits were immunized with an electrophoretically homogeneous preparation of thermostable HPAP, consisting of one single isozyme, pI 4.6.

The γ-globulin fraction of the antisera was obtained by triple precipitation with 50% $(NH_4)_2SO_4$ followed by dialysis against 0.01M K-phosphate buffer, containing 0.15 m NaCl, pH 7.4 (PBS), overnight at 4°C [2] and was used to isolate the IgG fraction of rabbit immunoglobulins by four different chromatographic methods.

The resulting preparations of antibodies and antiserum were tested by indirect enzyme immunoassay (EIA) and by the direct "sandwich" method.

For the indirect EIA test, EIA panels (Dynatech, Switzerland) adsorbed HPAP in a concentration of 20 μg/ml in 0.01 m Na-carbonate buffer (pH 9.6) in a volume of 100 μl per well

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