Free Radical Oxidation in Rat Brain during Chronic Stress and Pharmacological Regulation of This Process

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We studied free radical oxidation in the brain and blood serum from experimental animals exposed to chronic stress and receiving psychotropic drugs (phenazepam, Atarax, Fluanxol, and valerian). Chronic stress was accompanied by activation of free radical oxidation, which could be modulated by psychotropic drugs.

Key Words: *free radical oxidation; experimental stress; brain; psychotropic drugs*

Chronic stress is a cause of various nervous disorders, including anxiety, depression, phobia, *etc*. [1].

Activation of free radical oxidation (FRO) is one of the major reactions determining adaptation to stress. Prolonged stress exposure leads to hyperactivation of FRO, which results in damage to cell membranes and progression of pathological process [7]. Due to specific physiological characteristics, the nervous tissue is most susceptible to damage produced by FRO [2].

The impairment of oxidative metabolism is an important pathogenetic stage of stress-produced disturbances in the nervous system [1,2,5].

Psychotropic drugs are now extensively used in the therapy of these diseases [1,2]. Antioxidant activity of these drugs was confirmed in numerous experimental and clinical studies [2,6[. There is strong evidence that several antioxidant drugs have psychotropic activity [4].

Psychotropic drugs hold much promise in this respect. These compounds have intrinsic pro- or antioxidant activity and change the intensity of FRO in brain tissue via modulation of metabolic reactions.

Chemiluminescence (CL) recording is an informative method to study FRO in biological samples. CL is emission of light as a result of interaction between radicals. The intensity of FRO in tissue can be estimated from CL of its homogenate [10].

Here we studied FRO in the brain and serum from animals exposed to chronic stress and receiving psychotropic drugs. These drugs have similar pharmacological activity, but differ in the mechanism of action.

MATERIALS AND METHODS

Experiments were performed on 50 adult male outbred rats weighing 200-250 g. The animals were divided into groups (5 groups of 8 rats each; and 1 group of 10 rats). The rats were kept in cages and had free access to water and food.

The control group included 8 intact rats. Other animals were daily exposed to stress over 1 month. Stressed rats received NaCl (intramuscular injection) or water (intragastric administration, 10 rats), while others were treated with psychotropic drugs. We used benzodiazepine receptor agonist phenazepam (0.000025 g/kg intramuscularly); H_1 receptor antagonist Atarax (hydroxyzine hydrochloride, 0.001 g/kg intramuscularly); common valerian (2% valerian tincture, 0.1 g/kg intragastrically); and Fluanxol Depo (cis(Z)-flupentixol decanoate) affecting DA-1, DA-2, and 5-HT-2 receptors (0.002 g/kg intramuscularly, 1 time per 2 weeks). The doses of medicinal preparations corresponded to the mean daily therapeutic doses [1]. Forced swimming at 25°C for 25 min served as the model of stress [11,12].

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The severity of stress was estimated by changes in individual behavioral characteristics of animals in the open-field test. The following integral criteria were determined: locomotor index, orientation and exploratory activity, and emotional anxiety [3].

Study was performed in compliance with the Helsinki Declaration on the welfare of animals used for research.

The effects of test drugs on FRO in animals were evaluated by recording Fe^{2+}-induced CL in brain homogenate and serum. The animals were decapitated. The brain was removed on ice and washed with cold phosphatic buffer; brain samples were homogenized (1:5 w/v tissue-buffer ratio at 1000 rpm for 10 min) and the homogenate was filtered through a Capron filter. The homogenate (0.5 ml) was mixed with 19.5 ml phosphatic buffer and chemiluminescence was measured. The blood was sampled in centrifuge tubes, cells were pelleted by centrifugation at 3000 rpm for 15 min, and serum (0.5 ml) was mixed with phosphate buffer (19.5 ml) and studied on a chemiluminometer. Luminescence in samples was initiated with 1 ml 50 mM ferrous sulfate.

CL was recorded on a KhLM-003 device. Consistency of operation was estimated by recording luminescence of the secondary standard SFKhM-1. Luminescence of the standard (5.1×10^5 quanta per sec) was taken as an arbitrary unit. CL was recorded over 5 min. The total yield of CL was measured [10].

The content of thiobarbituric acid (TBA)-reactive substances was estimated in the reaction with TBA in the presence of trichloroacetic acid and reflected the intensity of lipid peroxidation in brain homogenate and serum. Spectrophotometry was performed at 532 nm [8]. Distilled water served as the control.

RESULTS

Chronic stress induced persistent behavioral changes in animals not receiving the test drugs. The observed changes corresponded to severe anxious depression. We revealed a decrease in orientation and exploratory activity and increase in emotional anxiety and locomotor index (Table 1). These parameters did not differ in animals treated intramuscularly with NaCl and receiving water intragastrically.

The behavioral pattern of valerian-receiving rats changed insignificantly, which corresponded to mild anxious depression. Treatment with Atarax prevented behavioral changes in animals. The integral criteria in rats of this group did not differ from the control. Phenazepam slightly decreased locomotor, orientation, and exploratory activities and reduced emotional anxiety in animals. Similar but more pronounced behavioral changes were observed in rats receiving Fluanxol Depo.

The intensity of FRO in rat brain underwent significant changes. In rats not receiving the test drugs, chronic stress produced an increase in CL of brain homogenate (by 2 times relative to the control) and serum (insignificantly, Table 2). The degree of pathological changes in CL of brain homogenate was 1.8-fold greater compared to the serum. The content of TBA-reactive substances increased in the brain and, to a lesser extent, in the serum.

In rats exposed to stress and receiving common valerian, the intensity of CL in brain homogenate increased by 1.5 times compared to the control. Similar changes were revealed in the content of TBA-reactive substances.

The intensity of CL in the brain of rats receiving Atarax was 1.14-fold higher than in intact animals. We revealed a slight increase in CL of brain homogenate in animals of the phenazepam group (by 1.16 times compared to the control). The content of TBA-reactive substances in the brain and serum and intensity of CL in the serum from rats receiving Atarax and phenazepam did not differ from the control.

Treatment with Fluanxol prevented the increase in CL of rat brain and serum. It should be emphasized that we found no differences between this group and controls. The content of TBA-reactive substances in

TABLE 1. Behavioral Parameters of Animals Exposed to Chronic Stress and Receiving Psychotropic Drugs ($M\pm m$, % of Control)

Group	Locomotor index	Orientation and exploratory activity	Emotional anxiety
Control	100.0±7.1	100.0±2.1	100.0±6.8
Stress	176.4±16.4*	80.50±1.77*	154.0±15.3*
Valerian	130.3±11.3*	84.19±3.90*	141.0±12.5*
Atarax	98.8±8.8	95.9±2.1*	118.0±10.1
Phenazepam	92.1±9.9*	93.30±1.85*	110.0±12.9
Fluanxol Depo	82.1±7.2*	91.20±1.99*	98.00±8.35

Note. Here and in Table 2: *$p<0.05$ compared to the control.

TABLE 2. Effect of Chronic Stress and Psychotropic Drugs on FRO in Rat Brain and Serum (*M*±*m*, % of Control)

Group	CL of brain homogenate	CL of serum	TBA-reactive substances in brain homogenate	TBA-reactive substances in serum
Control	100.0±2.3	100.00±2.25	100.0±3.4	100.0±5.5
Stress+	198.0±2.9*	110.0±6.2*	136.0±4.2*	105.0±4.9
Valerian	156.0±3.8*	104.0±5.4	116.0±3.9*	103.0±6.3
Atarax	114.0±2.6*	102.0±4.6	102.0±4.5	101.0±4.2
Phenazepam	116.0±4.5*	105.0±5.7	99.0±2.4	98.0±3.2
Fluanxol Depo	104.0±2.5	102.0±3.2	96.0±2.8	98.0±3.9

Note. +no differences between animals injected intramuscularly with NaCl and receiving water intragastrically.

the brain and serum from these rats did not differ from the control.

The differences between CL of brain homogenate and serum from stressed rats not receiving the test drugs was much more significant compared to intact animals and rats exposed to stress and treated with medicinal preparations. It could be related to a specific reaction of the brain to chronic stress.

Our results indicate that stress leads to FRO activation in the brain, increases emotional anxiety and locomotor activity, and decreases orientation and exploratory activity of animals. These changes serve as a manifestation of "stagnant" emotional excitation in the central nervous system (CNS) [9]. Psychotropic drugs prevented the development of these changes. The intensity of FRO and behavioral pattern of animals receiving the test drugs corresponded to normal or changed only insignificantly. It was probably associated with the effect of drugs on stress-limiting systems (phenazepam and valerian) or direct suppression of nervous function (Atarax and Fluanxol). This treatment resulted in a decrease in the stress reaction, which manifested in activation of FRO [7].

Inhibition of FRO in the brain corresponded to the degree of sedative and anxiolytic effects produced by the test drugs. These data reflect a relationship between psychopharmacological activity of drugs and their ability to modulate FRO in CNS (independently on pharmacodynamics of preparations).

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