Changes in Blood Biochemistry in Mice during Development of Experimental Depression

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Biochemical parameters of the plasma were studied in mice at different stages of the development of depression-like states in males after social defeats in 10 and 20 intermale confrontations (T10 and T20 victims, respectively). Glucose and cholesterol levels were increased in T10 victims in comparison with intact animals. In T20 victims the increase in glucose level was paralleled by an increase in total protein. T20 victims differed from T10 victims by lower catalase activity.

Key Words: *mice; intermale confrontations; glucose; cholesterol; enzymes*

It was previously shown that mice experienced long-term social stress in intermale confrontations were in a depression-like state after 20 days of social defeat [13]. The symptoms of pathological behavior in these animals and symptoms of depression in humans were very much similar. Depressive mice demonstrated a general behavioral deficiency in both individual and social behavior. At early stages of the development of experimental depression, stress caused pronounced mouse anxiety persisting for a long time. Depression was associated with disorders in the immune status of victims [3]; the involvement of brain transmitter systems, primarily of the serotoninergic system, in the development of depression was demonstrated [11]. Changes in activity of the transmitter systems depend on the duration of social stress, which indicates dynamic changes in the brain of mice, depending on the stage of disease development. Activity of the serotoninergic system increased at the early stages of experimental depression, but at the stage of deep depression decreased in brain structures specifically involved in the mechanisms of depression [11].

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We studied biochemical parameters of the plasma during the development of a depression-like state in mice experienced social defeat in 10 and 20 intermale confrontations. Glucose, total protein, cholesterol, lactate dehydrogenase (LDH), AlAT, and catalase activities were measured. These parameters were selected because they largely characterize changes in the main types of metabolism, intensity of cytolysis, and antioxidant reserves of the organism. Our aim was to identify peripheral markers of this model of social depression. We expected that it would help us to develop objective and simple criteria for evaluating the psychosomatic status during drug therapy of depressive patients.

MATERIALS AND METHODS

Experiments were carried out on adult male C57Bl/6J mice aged 2.5-3 months (24-26 g). The animals were bred and kept under standard vivarium conditions (Institute of Cytology and Genetics). Before the experiment the animals were kept in 36×23×12 cm cages, 6-8 animals per cage, and then placed in pairs into experimental cages (28×14×10 cm) divided with a transparent perforated wall. The animals were tested in 10-min intermale confrontations in the second half of the day (15.00-17.00) [12]. Victims (males experi-

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enced defeat during these confrontations) were selected. This status was fixed in daily repeated confrontations with aggressive partners. If attacks of the aggressor lasted more than 3 min, they were stopped by placing the wall between the mice. Biochemical parameters of blood plasma were analyzed in animals with 10- and 20-day experience of social defeat (T10 and T20 victims, respectively). Both groups of victims were compared with controls kept in individual cages for 5 days. Under these conditions social interactions were excluded, but the effect of social isolation did not develop.

Biochemical tests were performed 24 h after the last confrontation. The blood was collected after decapitation in heparinized tubes and the plasma was separated. Glucose, total protein, cholesterol, and activities of LDH and AlAT were measured using standard Biocon reagents. Catalase activity was measured in the ammonium molybdate test [5] in comparison with the reference sample (bovine liver catalase, 38.08 U/ml, ICN Biomedicals). The measurements were carried out on an automated Photometer-5010 (Boehringer Mannheim).

The results were statistically processed using Mann—Whitney's nonparametric *U* test and standard Statistica software.

RESULTS

Long-term confrontations between males cause social stress in mice and provoke depression-like pathological states in victims [13]. Experimental depression is paralleled by marked disorders in behavior and physiological status of victims.

Changes in blood biochemistry were detected. Confrontations increased plasma glucose levels in victims at early (T10: U=24.0, p<0.01) and later (T20: U=32.5, p<0.02) stages of the development of depression-like state (Fig. 1, *a*). During emotional stress, when activity of the sympathoadrenal system is high,

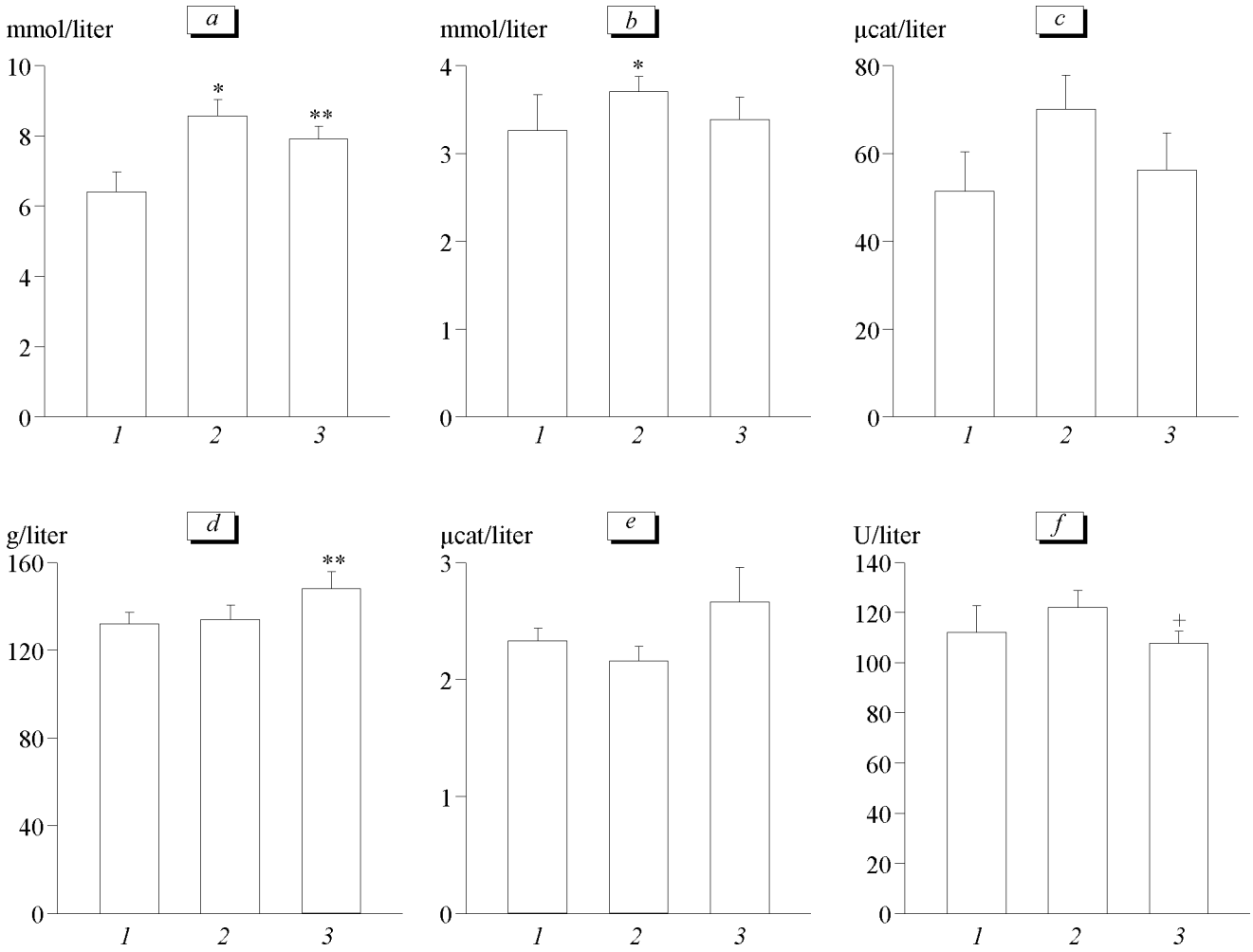

Fig. 1. Plasma content of glucose (*a*), cholesterol (*b*), lactate dehydrogenase (*c*), total protein (*d*), and activities of AlAT (*e*) and catalase (*f*) in male C57Bl/6J mice with depression-like state. *1*) intact controls; *2*) animals with 10-day experience of social defeats (T10); *3*) animals with 20-day experience of social defeats (T20). *p<0.01, **p<0.05 compared to control. ^{+}p<0.05 compared to T10.

glucose consumption increased, because it is the main source of energy for nerve cells. The detected increase in glucose levels in T10 and T20 groups under conditions of long-term emotional distress can result from high plasma corticosterone concentration [4]. Corticosteroids activate glycogenolysis; glucose is delivered to organs, whose function is vitally important in stress. Increased secretion of glucocorticoids can determine increased plasma cholesterol level, which can be regarded as a compensatory reaction (in response to increased consumption of the substrate for the synthesis of adrenocortical hormones). Moreover, stress is associated with increased consumption of cholesterol as a structural component of cell membranes. There are clinical data on hypercholesterolemia in patients with bipolar disorders [9]. We observed an increase in plasma cholesterol level after 10 days of confrontations (Fig. 1, *b*: U=26.0, p<0.01).

Our findings indicate that the increase in blood glucose level was not paralleled by significant changes in LDH activity (glycolytic conversion of glucose, LDH catalyzes oxidation of lactate into pyruvate) (Fig. 1). On the other hand, in T10 victims LDH activity slightly increases in comparison with the control (U=42.5, p<0.09; Fig. 1, *c*). This is in line with our previous results on LDH activity in blood lymphocytes: enzyme activity significantly increased in T10 victims and decreased to the control level at the stage of manifest disease [3].

The increase in blood glucose level in T20 victims was paralleled by an increase in total protein level (U=38.5, p<0.05; Fig. 1, *d*). Hence, long-term social confrontations led to mobilization of both energy (glucose) and structural (protein) resources. These functional changes are characteristic of urgent adaptation, forming under conditions of emotional stress [7,8].

However, the adaptive reaction in victims was paralleled by destructive processes associated, presumably, with activation of LPO, the peripheral component of the stress-realizing system (and antioxidant system of organs and tissues, regulating LPO level, acting as the stress-limiting system) [7]. Antioxidant enzymes (peroxidases, superoxide dismutase, and catalase) are the most important components of this system. Decreased activities of these enzymes under conditions of LPO activation promote nonspecific pathological effects of stress [2,10]. For example, impaired structural integrity and enhanced permeability of biological membranes led to the release of cell contents into the blood. Increased AlAT activity in the blood reflects the intensity of cytolytical processes, primarily in the liver. In our experiments plasma levels of AlAT and catalase after 10 days of confrontations were close to the control (Fig. 1, *e*, *f*). However, catalase activity decreased in T20 victims (U=33.5, p<0.05), which indicated impairment of antioxidant reserves with the progress of experimental depression (Fig. 1, *f*). These data suggest intensification of LPO during long-term intermale confrontations. This is in line with previous studies demonstrating similar accumulation of LPO products in the blood of humans and animals under conditions of emotional stress [2,7] and in patients with neuropsychiatric pathology [1] and bipolar disorders [6].

Hence, the development of experimental depression after 10-20 confrontation between males is associated with shifts in biochemical parameters of the blood (increase of glucose, total protein, and cholesterol concentrations). These changes are characteristic of urgent adaptation [7]. However, the decrease in catalase activity on day 20 of the experiment indicates intensification of LPO processes and exhaustion of the enzyme components of the antioxidant system [10].

Our findings indicate that the development of depression-like state caused by long social stress involves shifts in nonspecific biochemical parameters of the blood. Increased concentrations of glucose and cholesterol and slight increase in LDH activity were detected in victims with 10-day experience of defeats. Catalase activity, reduced significantly in T20 victims in comparison with T10 victims, can be regarded as a peripheral marker of depression-like state developing under conditions of this model of chronic social stress.

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