PRIMATOLOGY

Hypothalamic-Pituitary-Adrenal System and Enzymes of the Glutathione-Dependent Antioxidant System during Stress and Aging

N. D. Goncharova, A. V. Shmaliy, V. Yu. Marenin*, and S. A. Smelkova

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Activity of glutathione-dependent antioxidant enzymes (glutathione peroxidase, glutathione reductase, and glutathione transferase) in blood erythrocytes from female *Macaca mulatta* (6-8 and 20-26 years) was measured during activation of the hypothalamic-pituitary-adrenal system under conditions of acute psychoemotional stress (2-h restraint stress). Glutathione reductase activity increased during stress, depended on the time of day, and strongly correlated with blood corticosteroid level. Circadian variations in stress reactivity of glutathione reductase during aging were accompanied by similar changes in stress reactivity of the hypothalamic-pituitary-adrenal system.

Key Words: *glutathione reductase; corticosteroids; acute psychoemotional stress; aging; monkeys*

Enzymes of the glutathione-dependent antioxidant system, glutathione peroxidase, glutathione reductase (GR), and glutathione transferase, play an important role in cell protection from overproduction of reactive oxygen species, including superoxide radical and its derivatives (H_2O_2, hydroxyl radical, and peroxynitrite). Moreover, they modulate intracellular redox homeostasis by regulating the concentrations of reduced and oxidized glutathione. Substrate regulation of enzymes in the glutathione-dependent antioxidant system was described [3,4, 6,8]. However, hormonal regulation of enzyme activity is poorly understood.

Laboratory of Endocrinology, Institute of Medical Primatology, Russian Academy of Medical Sciences, Sochi; *Sochi State University of Tourism and Health Resort Business. ***Address for correspondence:*** ndgoncharova@mail.ru. N. D. Goncharova

Little is known about the effect of the hypothalamic-pituitary-adrenal system (HPAS) on age-related variations in enzyme activity of the glutathione-dependent antioxidant system. Most studies were performed on cultured cells or laboratory rodents after treatment with corticosteroids in pharmacological doses [10,12]. However, hormonal regulation and secretion of dehydroepiandrosterone sulfate (DHEA-S) significantly differ in primates and rodents [1,7,9]. Therefore, laboratory primates are a more suitable model to study hormonal regulation and hormonal replacement therapy.

Enzyme activity of the glutathione-dependent antioxidant system in blood erythrocytes from female *Macaca mulatta* of different age was studied during activation of HPAS under conditions of acute psychoemotional stress (2-h immobilization) at 9.00 and 15.00.

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MATERIALS AND METHODS

Experiments were performed on 7 pubertal young (6-8 years) and 7 old (20-26 years) female rhesus monkeys (*Macaca mulatta*) weighing 4.5-6.0 kg. The animals were maintained in cages at the nursery of the Institute of Medical Primatology. During the study, the monkeys were housed in individual metabolic cages at controlled temperature (20-25°C) and light/dark cycle (daytime 6.00-18.00). All experiments were conducted in the summer-autumn period, which coincided with the absence of ovarian and menstrual cycles in animals. The monkeys received standard pelleted food and water *ad libitum*. They were additionally given bread, boiled egg, fresh vegetables, and fruits. The animals were adapted to environmental conditions in metabolic cages and procedure of blood sampling for not less than 4 weeks before the study.

The monkeys were subjected to acute restraint stress for 2 h ("soft" immobilization in metabolic cages). During restraint stress, a movable posterior wall was moved to press the animal to the anterior wall of the metabolic cage. The body and limbs were not firmly fixed. The animals were stressed 2 times a day at 9.00 and 15.00. Repeated stress exposures were performed at a 2-week interval. Blood samples (2.5-3.0 ml) were taken from the cubital or femoral vein of fasting monkeys before and 15, 30, 60, 120, and 240 min after the start of immobilization. Heparin was used as the anticoagulant. Blood samples were centrifuged at 2000*g* and 4°C. The plasma and erythrocytes were separated and stored at -70°C. Erythrocytes were hemolyzed immediately before enzyme assay. Activities of glutathione peroxidase (EC 1.11.1.9), GR (EC 1.6.4.2), and glutathione transferase (EC 2.5.1.18) in hemolysates were measured by kinetic spectrophotometric methods [5]. Glutathione peroxidase activity was expressed in mmol reduced glutathione/min per mg total erythrocyte protein. GR activity was expressed in nmol NADPH/min per mg total erythrocyte protein. Glutathione transferase activity was expressed in μmol glutathione-1-chloro-2,4-dinitrobenzene conjugate/min per mg total erythrocyte protein. The concentration of corticosteroids (cortisol and DHEA-S) in blood plasma was measured by enzyme immunoassay using standard reagents for study of cortisol (AlkorBio) and DHEA-S (DSL). Plasma glucose concentration was estimated by the glucose oxidase method. The variation coefficient of hormone concentration in one test with each blood sample did not exceed 10%. This coefficient in various tests did not exceed 15%.

The data were processed by analysis of variance (ANOVA) and correlation analysis.

RESULTS

The increase in plasma cortisol concentration in young animals 120 min after stress exposure at 15.00 was much more significant compared to that at 9.00 (Table 1). The area under cortisol concentration curve after stress at 15.00 tended to increase compared to that at 9.00 (Table 1). Young animals were characterized by circadian differences in the increase in DHEA-S concentration. DHEA-S concentration in these monkeys tended to increase more significantly after stress at 15.00 (Table 2). Similar results were obtained previously with young female rhesus monkeys [2]. These data illustrate the existence of circadian variations in stress reactivity of HPAS. Circadian variations in stress reactivity of the adrenal cortex are probably related to greater stress reactivity of adrenocorticotropic hormone at 15.00 [2].

GR activity increased more significantly after stress at 15.00 than at 9.00 (Table 3).

A strong correlation was found between changes in GR activity and corticosteroid concentration in young stressed animals. The coefficients of correlation between activity of GR and concentrations of

TABLE 1. Immobilization-Induced Changes in Cortisol Concentration in Peripheral Blood Plasma from Female *Macaca mulatta* (nmol/liter, *M*±*m*)

Time of day	Age, years	Time after the start of immobilization, min						Area of the response, μmol/liter/min
		0	15	30	60	120	240	
9.00	6-8	930±50	1080±90	1280±85	1330±57	1360±50	1060±80	230±10
	20-26	810±55	996±85	1020±70	1100±66	1340±160	1000±80	250±20
15.00	6-8	730±20	1023±40	1110±48	1332±60	1714±130	1369±170	250±17
	20-26	720±60*	940±60	1000±60	1170±140	1240±150*+	1010±110	230±20

Note. *$p<0.01$ compared to the corresponding parameter at 9.00; +$p<0.05$ compared to young animals.

TABLE 2. Immobilization-Induced Changes in DHEA-S Concentration in Peripheral Blood Plasma from Female *Macaca mulatta* of Different Age (nmol/liter, *M*±*m*)

Time of day	Age, years	Time after the start of immobilization, min					Area of the response, μmol/liter/min
		0	30	60	120	240	
9.00	6-8	660±120	780±160	800±130	850±110	734±140	140±20
	20-26	130±50**	170±70*	165±60**	190±70**	290±110*	35±10**
15.00	6-8	530±90	640±90	850±160	1140±240	680±130	150±3
	20-26	60±30**	90±30***	95±40**	100±30**	60±20+***	16±5***

Note. *p<0.05, **p<0.01, and ***p<0.001 compared to young animals; +p<0.05 compared to the corresponding parameter at 9.00.

cortisol and DHEA-S after stress at 15.00 were 0.93±0.08 and 0.85±0.08, respectively. Stress reactivity of HPAS and activity of GR decreased in the morning time. These changes were accompanied by a decrease in the correlation coefficient, which reflected the relationship between HPAS and GR (r=0.50±0.05 for cortisol and GR; r=0.51±0.50 for DHEA-S and GR).

The strict correlation between stress-induced changes in corticosteroid concentration and GR activity reflects the dependence of GR activity on HPAS function. Corticosteroids increase erythrocyte GR activity, which is probably associated with the stimulatory effect of these hormones on blood glucose concentration. For example, a strong correlation was found between changes in GR activity and blood glucose concentration after acute stress at 15.00 (r=0.67±0.75). Moreover, a correlation was observed between glucose concentration and cortisol/DHEA-S ratio (r=0.80±0.76). The increase in blood glucose concentration is followed by the rise in erythrocyte glucose concentration. These changes are accompanied by activation of glucose-6-phosphate dehydrogenase, which serves as a major source of reduced NADPH (essential coenzyme of GR). A stimulatory effect of corticosteroids on GR activity is probably related to an increase in the synthesis of reduced NADPH.

Circadian variations in stress reactivity of HPAS and HR typical of young animals were not observed in old monkeys (Tables 1-3). Corticosteroid concentration and GR activity in old animals changed similarly after stress in the morning and evening. Hence, aging was accompanied by reduction of circadian rhythms in stress reactivity of corticosteroids and GR. Moreover, reactivity of HPAS and GR in the morning was sometimes higher than in the evening. DHEA-S concentration in old animals was higher 240 min after stress at 9.00 than at 15.00 (Tables 1 and 2). The area of the response for cortisol and DHEA-S tended to increase (Table 2). GR activity tended to increase more significantly 240 min after immobilization at 9.00 that at 15.00 (Table 3). Despite age-related abnormalities in the circadian rhythm of HPAS and GR in stressed animals, a strong correlation was found between stress-induced changes in corticosteroid concentration and GR activity (r=0.80±0.08 and 0.63±0.07 for cortisol and GR at 15.00 and 9.00, respectively; r=0.88±0.09 and 0.70±0.06 for DHEA-S and GR at 15.00 and 9.00, respectively). High correlation coefficients for changes in GR activity and cortico-

TABLE 3. Immobilization-Induced Changes in GR Activity in Blood Erythrocytes from *Macaca mulatta* (nmol NADPH/mg protein/min, *M*±*m*)

Time of day	Age, years	Time after the start of immobilization, min				
		0	30	60	120	240
9.00	6-8	0.56.±0.05[1]*	0.59±0.05	0.70±0.08	0.70±0.18	0.69±0.09
9.00	20-26	0.55±0.06	0.58±0.05	0.64±0.09	0.63±0.14	0.90±0.10*
15.00	6-8	0.30±0.07	0.50±0.09	0.80±0.06**	0.84±0.10**	0.94±0.20*
15.00	20-26	0.52±0.10	0.51±0.10	0.66±0.26	0.68±0.08	0.60±0.10

Note. *p<0.05 and **p<0.01 compared to the pre-immobilization parameter (0 min); +p<0.05 compared to young animals.

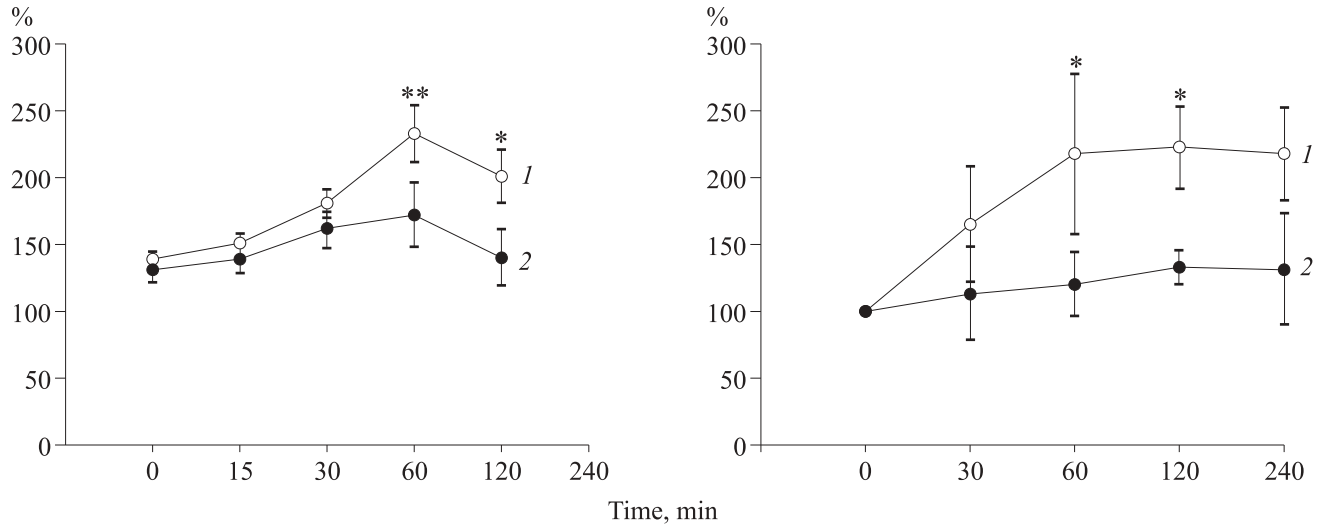

Fig. 1. Cortisol concentration in peripheral blood plasma (*a*) and GR activity in erythrocytes (*b*) from female *Macaca mulatta* after immobilization at 15.00 (% of the basal level, $M \pm m$). 6-8 years (*1*) and 20-26 years (*2*). $^{*}p<0.05$ and $^{**}p<0.01$ compared to old animals.

steroid concentration in old animals indicate that HPAS plays an important role in the regulation of GR stress reactivity (despite a sharp decrease in basal DHEA-S concentration and reduction of circadian rhythms in cortisol production, Table 2).

Age-related differences in stress reactivity of HPAS and GR were revealed during immobilization of animals at 15.00 (Tables 1 and 2). The stress-induced increase in cortisol concentration and GR activity at various periods after stress exposure in young animals was much more significant than in old monkeys (Fig. 1).

Activities of glutathione peroxidase and glutathione transferase remained practically unchanged during stress and aging.

Age-related abnormalities in stress reactivity of GR are of considerable pathophysiological importance for function of erythrocytes and whole organism. The less pronounced increase in GR activity in old animals during acute stress can impair generation of reduced glutathione, ratio of reduced glutathione to oxidized glutathione, and homeostasis of erythrocytes. These changes impair reliability of the antioxidant defense system, increase the concentration of reactive oxygen species in erythrocytes, and lead to activation of lipid peroxidation (LPO). Activation of LPO can be accompanied by hemolysis of erythrocytes and decrease in the reliability of oxygen transport to tissues with increased oxygen demands.

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