PRIMATOLOGY

Effect of Aging on Stress Reactivity of the Adrenal Cortex in Laboratory Primates. Dependence on the Time of Day

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Age-specific differences in the reaction of the hypothalamic-pituitary-adrenal system to acute psychoemotional stress (immobilization) was studied in female rhesus macaques aged 6-8 and 20-27 years at different time of the day. The reactivity of the hypothalamic-pituitary-adrenal system during immobilization at 15.00 was lower in old animals, while at 9.00 there were no age-specific differences or the reactivity was higher in old animals.

Key Words: *stress; aging; hypothalamic-pituitary-adrenal system; monkeys*

The effects of aging on stress reactivity of the hypothalamic-pituitary-adrenal system (HPAS) are little studied. These studies were carried out mainly on rodents [7-10]. However, HPAS functioning in rodents normally, in stress, and during aging differs significantly from that in humans [3,11]. The functioning of HPAS under basal conditions and during aging is similar in humans and laboratory primates [1,2,4]. For example, circadian rhythm disturbances of the basal levels of hydrocortisone (F) during aging were observed in humans [6,12,13] and monkeys [3]. We studied age-specific differences in stress reactivity of HPAS in female rhesus macaques at different time of the day.

MATERIALS AND METHODS

Experiments were carried out on 11 young adult (6-8 years) and 11 old (20-27 years) healthy female rhesus macaques (*Macaca mulatta*) living in Breeding Center of Institute of Medical Primatology. Experiments were carried out in summer (June-August) 2003 and 2004. Animals living in groups in cages were placed during the experiment into individual metabolic cages in isolated rooms with controlled illumination (light from 7.00 to 19.00) at 20-26°C. The animals received balanced nutrition (briquette fodder made using Altromin technology, fresh vegetables and fruit, water *ad libitum*). Before the experiment the animals had a course of adaptation to individual cages (for at least 4 weeks) and blood collection procedure.

In 2004 experiments were carried out on 4 young (6.3±0.3 years) and 4 old (22.8±0.8 years) rhesus macaques weighing 5.0±0.3 and 5.6±0.5 kg, respectively. The animals were twice subjected to acute stress (non-rigid immobilization in metabolic cages) for 2 h. Stress exposure was carried out at different time of the day at 2-week interval, at 9.00 or 15.00. Blood samples were collected before and 15, 30, 60, 120, and 240 min after immobilization.

In 2003 the study was carried out on 7 young (7.4±0.15 years) and 7 old (21.6±0.45 years) rhesus female macaques weighing 5.0±0.3 kg and 6.6±0.8 kg, respectively, which were subjected to non-rigid 2-h immobilization in metabolic cages. Immobilization was started at 15.00. Blood samples

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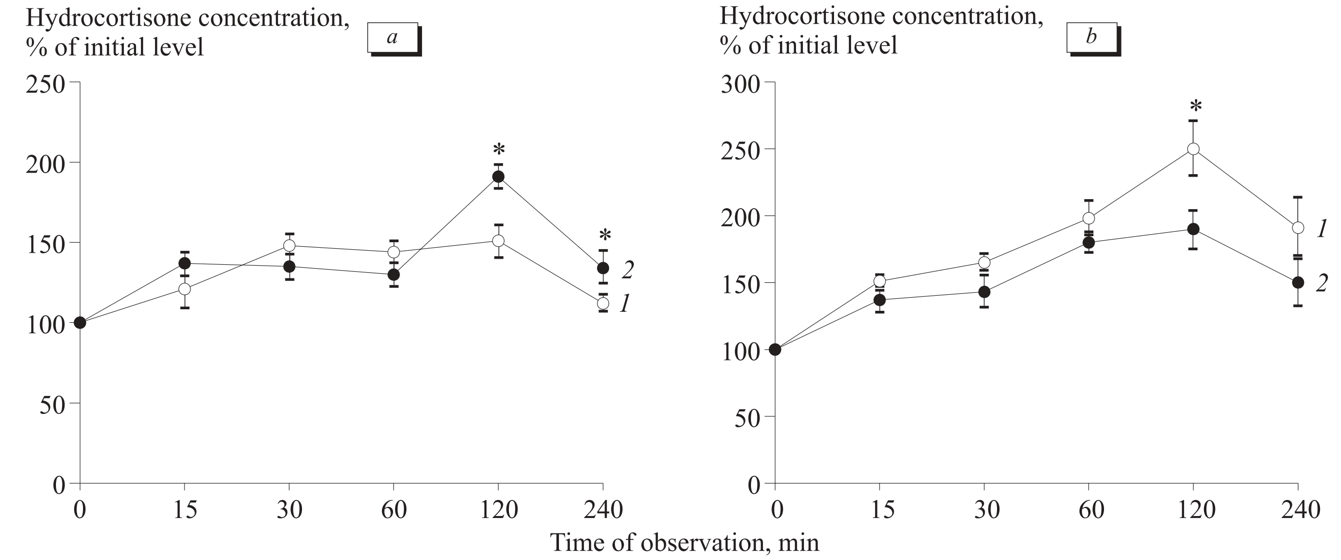

Fig. 1. Changes in hydrocortisone concentrations in the peripheral blood plasma of *Macaca mulatta* females of different age during acute stress exposure (immobilization) started at different time of the day. Here and in Fig. 2: *a*) exposure started at 9.00; *b*) at 15.00; *1*) 6-8 years; *2*) 20-27 years. *$p<0.05$ compared to the corresponding parameter in old animals.

were collected before and 15, 30, 60, 120, and 240 min after immobilization.

The blood was collected from the ulnar or femoral veins and stabilized with EDTA. Blood was immediately centrifuged at 2000*g* and 4°C. Plasma was separated and stored at -70°C until hormone assay. Hormones were measured no later than 1 month after blood collection.

The content of F was measured in all blood samples, ACTH in samples collected before and 15, 30, and 60 min after immobilization. ACTH and F concentrations were measured by enzyme immunoassay using commercial kits (DSL for ACTH and AlkorBio). Coefficient of ACTH variations within one and different tests did not exceed 10 and 15%, respectively, coefficient of variations for F 10 and 12%, respectively.

The results were statistically processed using Student's *t* test.

RESULTS

No statistically significant differences in basal F levels and increase of F level in response to stress were observed during the morning hours (Table 1). However, expression of F level in percent of the initial level showed a significantly higher level of F in old animals in comparison with young ones 120 and 240 min after the beginning of immobilization (Fig. 1).

Immobilization of the same animals in the evening resulted in a significantly more pronounced increase of F level in young animals in comparison with old ones 120 min after the start of acute stress exposure (Table 1, Fig. 1). The area under the curve presenting changes in blood F level in response to stress exposure in young animals also significantly increased (Table 1). Similar changes were observed when elevation of F content was expressed in per-

TABLE 1. Changes in F Concentrations (nmol/liter, *M*±*m*) in Peripheral Blood Plasma of *Macaca mulatta* Females of Different Age in Response to Acute Stress Exposure Started at Different Time of the Day (9.00 and 15.00) (Experiment 2004)

Hours, age		Time after immobilization, min						Response area, mmol/liter×min
		0	15	30	60	120	240	
9:00	6-8 years	935±30	1130±90	1380±110	1340±100	1400±50	1050±70	240±20
	20-26 years	800±80	1040±80	1080±110	1160±80	1530±70	1070±90	245±20
15:00	6-8 years	715±20	1080±17	1180±60	1420±50	1810±40*+	1365±150	270±10*
	20-26 years	740±50	1020±50	1060±17	1340±60	1440±85	1160±100	230±7

Note. Four animals per age group. *$p<0.01$ compared to corresponding values in old animals subjected to immobilization at 15.00; +$p<0.001$ compared to immobilization at 9.00.

TABLE 2. Changes in F Concentrations (nmol/liter, *M*±*m*) and ACTH (pg/ml, *M*±*m*) in Peripheral Blood Plasma of *Macaca mulatta* Females of Different Age in Response to Acute Stress Exposure Started at 15.00 (Experiment 2003)

Hours, age	Time after immobilization, min						Response area
	0	15	30	60	120	240	
F, nmol/liter							
6-8 years	660±80	800±40	850±40	1010±50	1050±30	690±60	210±3 mmol/liter×min
20-26 years	790±28	840±30	880±24	950±25	810±40**	585±40	200±7 mmol/liter×min
ACTH, pg/ml							
6-8 years	27±8	50±10	65±9	100±20	—	—	3520±370 pg/ml×min
20-26 years	39±10	51±10	44±6	30±3**	—	—	2600±240* pg/ml×min

Note. Seven animals per age group. *$p<0.05$, **$p<0.001$ compared to the corresponding parameter in young animals.

cents of the initial value (Fig. 1). The data of experiments carried out in 2004 coincided with findings of 2003 (Table 2).

Comparative analysis of changes in ACTH level in the peripheral blood plasma of animals of different age in response to stress was carried out (Fig. 2, Table 2). The level of ACTH was higher in old animals than in young ones during immobilization at 9.00 (Fig. 2). The area under the curve presenting ACTH changes in response to immobilization was also larger in old monkeys (3160±580 pg/ml×min *vs.* 1770±300 pg/ml×min in young monkeys, $p<0.05$). On the other hand, the increase of ACTH level in response to immobilization at 15.00 was significantly higher in young animals (Fig. 2). The area under ACTH response curve in young females was also larger than in old animals (3480±80 pg/ml×min *vs.* 2270±500 pg/ml×min, respectively, $p<0.05$). Similar age-specific differences in ACTH reaction to stress were observed in experiments of 2003 (Table 2).

The results attest to the leading role of age-specific differences in the adenohypophyseal reactivity (ACTH secretion) to stress in animals with age-specific changes in the adrenocortical reaction to acute stress exposure. It seems that aging is associated with disorders in the circadian rhythms of the pituitary reactivity to stress, resulting in age-specific changes in the adrenocortical stress reactivity, detected in our study. This phenomenon can

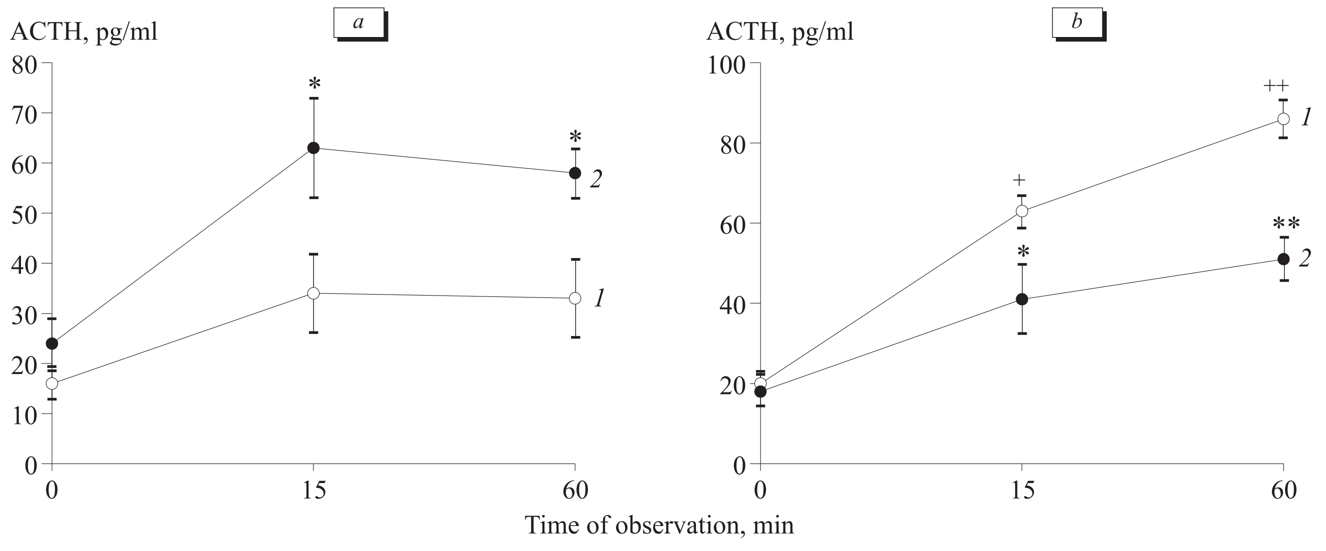

Fig. 2. Changes in ACTH concentrations in the peripheral blood plasma of *Macaca mulatta* females of different age during acute stress exposure (immobilization) started at different time of the day. *$p<0.05$, **$p<0.001$ compared to young animals; $^+p<0.01$, $^{++}p<0.001$ compared to the values during immobilization at 9.00.

be due to impairment of the hypothalamic suprachiasmatic nuclei determining the majority of circadian rhythms in old animals. The development of degenerative changes in the hypothalamic suprachiasmatic nuclei during aging was previously reported [5,14].

Hence, age-specific differences in stress reactivity of the HPAS depend on the time of the day: at 15.00 HPAS stress reactivity is significantly higher in young animals, while in the morning (at 9.00) there are no significant differences between young and old animals or old animals exhibit higher reactivity.

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