# PRIMATOLOGY

# Repeated Moderate Stress Stimulates the Production of Dehydroepiandrosterone Sulfate (DHEAS) and Reduces Corticosteroid Imbalance in Old *Macaca Mulatta*

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Young (6-8 years) and old (21-30 years) *Macaca mulatta* females were subjected to gentle immobilization (2 h daily at 15.00) for 10 days. Blood specimens were collected before the exposure and 15, 30, 60, 120, 240 min and 24 h after the beginning of exposure on days 1, 3, and 10. The adrenocortical reaction to stress was maximum on day 1 in all animals. The increase of cortisol (F) and dehydroepiandrosterone sulfate (DHEAS) concentrations in young monkeys decreased on days 3 and 10, DHEAS drop being less pronounced in comparison with F, as a result of which F/DHEAS molar concentration ratio changed negligibly. In old monkeys the basal DHEAS levels were lower, while the F/DHEAS ratio was higher than in young animals. Repeated immobilizations inhibited F elevation on day 3, caused no changes in DHEAS reaction, led to increase of basal DHEAS levels and to a reduction of F/DHEAS ratio on days 2, 3, 4, 10, 11. Hence, chronic moderate stress stimulated the production of DHEAS and reduced the corticosteroid imbalance in old monkeys.

**Key Words:** *dehydroepiandrosterone sulfate; cortisol; stress; aging; Macaca mulatta*

The pathogenetic role of mental stress in the development of many mental and mental-somatic diseases is well known; the incidence of these diseases increases sharply with aging. Hypercortisolemia and low secretion of adrenal androgens (dehydroepiandrosterone and dehydroepiandrosterone sulfate (DHEAS) are assumed to play an important role in the negative effects of stress [3,4,9]. These androgens partially neutralize the toxic effects of excessive glucocorticoids [1,10,13]. The reactions of the adrenal cortex to acute and chronic stress have been studied in aging rodents, laboratory primates, elderly and senile humans [2,4,6,7,12,14]. However, the rodent adrenals do not produce DHEAS [1,11], and DHEAS secretion has not been studied in the majority of clinical and experimental studies on primates [7,12,14].

We studied the age-associated reactions of the adrenal cortex to multiple repeating moderate mental stress exposure (nonrigid immobilization) in *Macaca mulatta* females.

## MATERIALS AND METHODS

Experiments were carried out on 8 young (6-8 years) and 8 old (21-30 years) *Macaca mulatta* females, kept at breeding center of Institute of Medical Primatolo-

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gy. Experiments were carried out in summer (June-August), when ovarian cycles were not characteristic of this species of laboratory primates. The animals, living in groups in corals or cages, were put during the experiments into individual metabolic cages, placed in an isolated room with natural light (usually from 7.00 till 19.00). Ambient temperature varied from 20 to 26°C. The animals received balanced diets (Lage briquetted fodder manufactured by the Altromin technology), water *ad libitum*, fresh vegetables and fruits. Before experiments the animals were adapted to individual cages and blood collection procedure for 4 weeks.

The animals were subjected to mental stress (2-h daily nonrigid immobilization in metabolic cages) in the afternoon (at 15.00) over 10 days. Immobilization procedure was described previously [6]. Blood specimens were collected before immobilization (day 0), on days 1, 3, 10 of immobilization, and 15, 30, 120, 240 min and 24 h (1440 min) after the beginning of immobilization.

Blood specimens were collected from the ulnar or femoral veins with heparin as the anticoagulant. Blood was directly centrifuged at 3000g and 4°C. Plasma was stored at -50°C before hormone analysis. Plasma levels of cortisol (F) and DHEAS were measured by enzyme immunoassay with commercial kits (Adaltis for DHEAS and AlcorBio for F). Coefficients of variations in F and DHEAS levels for one test and different tests did not exceed 10 and 15%, respectively.

The results were statistically processed by Student's *t* test and ANOVA.

## RESULTS

The most marked elevation of F secretion in young and old animals in response to stress was recorded on day 1 (Fig. 1). The peak of F concentration was recorded in 60 min after the start of exposure in young animals (170±8%) and in 120 min in old animals (165±20%). The hormone concentration normalized after 240 min in animals of both age groups.

On day 3, the concentration of F in young animals reached the peak (143±9%) in response to immobilization sooner than on day 1 (after 30 min) and was significantly lower than on day 1 (1076±48 *vs.* 1310±60 nmol/liter, $p<0.001$). The hormone concentrations 30, 60, 120, 240, and 1440 min after the start of the exposure were also significantly lower (Fig. 1). In old animals, F concentration also reached the peak (136±14%) sooner than on day 1 (after 60 min). The maximum F values just tended to decrease (870±87 *vs.* 1050±120 nmol/liter; $p<0.05$), but after 120 and 1440 min were significantly lower than in response to immobilization 1 ($p<0.05$).

**TABLE 1.** Changes in F/DHEAS Molar Concentration Ratio in the Peripheral Blood in *Macaca Mulatta* of Different Age in Response to Repeated Stress for 10 Days (Daily 2-h Immobilization) (*M±m*)

| Immobilization | Time after the start of immobilization, min | | | | | | |
|---|---|---|---|---|---|---|---|
| | 0 | 15 | 30 | 60 | 120 | 240 | 1440 |
| | F/DHEAS | | | | | | |
| Young animals (6-8 years) | | | | | | | |
| 1 | 0.34±0.04 | 0.38±0.05 | 0.35±0.06 | 0.32±0.06 | 0.29±0.04 | 0.23±0.05 | 0.26±0.08 |
| 3 | 0.40±0.10 | - | 0.44±0.09 | 0.33±0.08 | 0.33±0.10 | 0.26±0.07 | 0.25±0.07 |
| 10 | 0.32±0.03 | 0.32±0.03 | 0.29±0.04 | 0.28±0.02 | 0.28±0.03 | 0.26±0.02 | 0.30±0.03 |
| Old animals (21-30 years) | | | | | | | |
| 1 | $1.60\pm0.25^{+}$ | $1.91\pm0.30^{+++}$ | $1.93\pm0.28^{+++}$ | $1.24\pm0.20^{+}$ | $0.88\pm0.10^{+o}$ | $0.47\pm0.09^{+oo}$ | $0.78\pm0.26^{+o}$ |
| 3 | $1.50\pm0.20^{+}$ | □ | $1.42\pm0.15^{+}$ | $1.34\pm0.30^{+}$ | $0.83\pm0.30$ | $0.59\pm0.10^{+oox}$ | $0.61\pm0.10^{oox}$ |
| 10 | $0.91\pm0.09^{*+++\#}$ | $0.98\pm0.10^{*+x}$ | $1.00\pm0.09^{*****+++x}$ | $0.99\pm0.08^{+++x}$ | $0.99\pm0.10^{+++x}$ | $0.77\pm0.08^{+++x}$ | $0.76\pm0.10^{+++x}$ |

**Note.** $^{*}p<0.05$, $^{***}p<0.001$ in comparison with the values in response to immobilization 1; $^{+}p<0.05$, $^{+++}p<0.001$ in comparison with young animals; $^{o}p<0.05$, $^{oo}p<0.01$ in comparison with the initial level (0); $^{x}p<0.05$ in comparison with the initial level (0) on day 1; $^{\#}p<0.05$ in comparison with the values in response to immobilization 3.

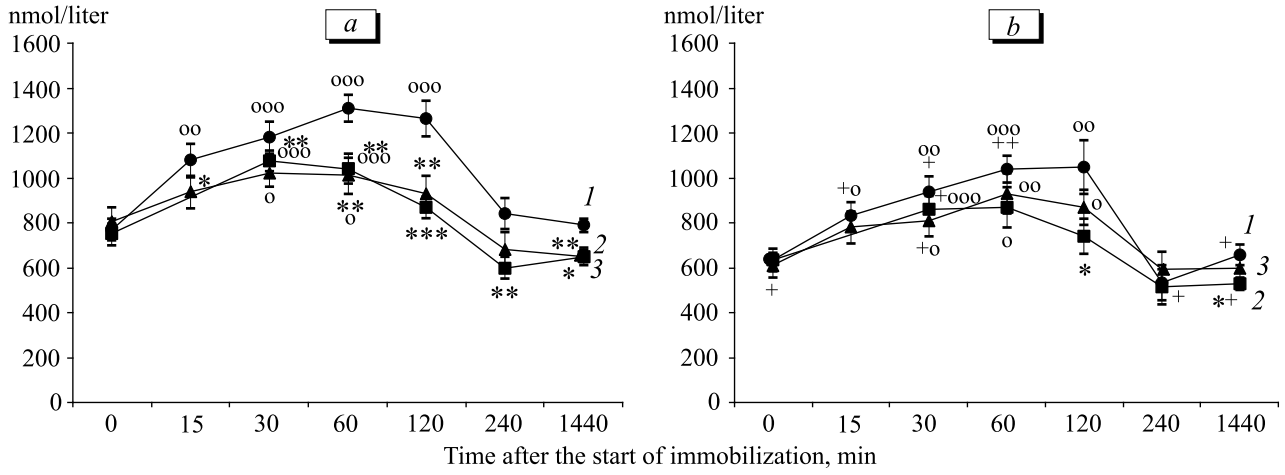


**Fig. 1.** Changes in peripheral blood F concentrations in *M. mulatta* of different age in response to repeated stress exposure for 10 days. **p*<0.05, ***p*<0.01, ****p*<0.001 in comparison with the response to immobilization 1; $^{+}p$<0.05, $^{++}p$<0.01 in comparison with young animals; $^{o}p$<0.05, $^{oo}p$<0.01, $^{ooo}p$<0.001 in comparison with the initial level (0). Here and in Fig. 2: *a*) 6-8 years; *b*) 21-30 years; *1*) day 1; *2*) day 3; *3*) day 10.

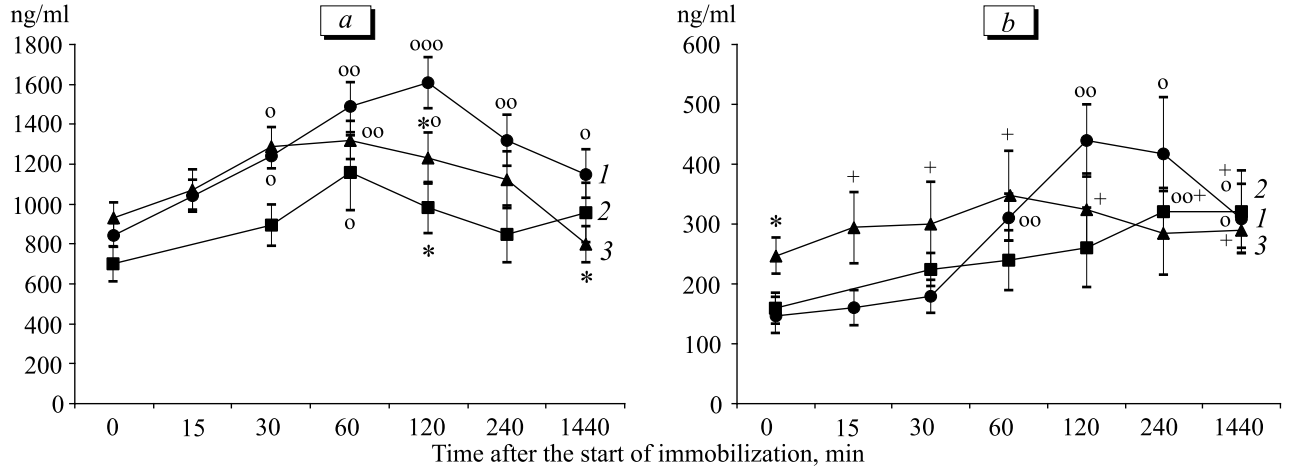


**Fig. 2.** Changes in peripheral blood DHEAS concentrations in *M. mulatta* of different age in response to repeated stress exposure for 10 days. **p*<0.05 in comparison with the response to immobilization 1; $^{o}p$<0.05, $^{oo}p$<0.01, $^{ooo}p$<0.001 in comparison with the initial level (0), $^{+}p$<0.05 in comparison with the initial level (0) on day 1.

On day 10, F concentration in young animals reached the maximum level (127±6%) in 30 min, in old ones in 60 min (152±10%) after the start of immobilization (Fig. 1). The maximum F values and the values 15, 60, 120, and 1440 min after the beginning of stress exposure in young animals were significantly lower than the values on day 1 and the same as on day 3. Old animals developed no appreciable differences in the reaction of F secretion to immobilization sessions 1 and 3.

The area under the curve presenting the time course of F concentrations from min 0 to min 240 (response area) on days 3 and 10 was significantly smaller than the F response area on day 1 (220,350±11,700 and 222,670±17,000 nmol/liter×min, respectively, on days 3 and 10 *vs.* 272,730±16,000 nmol/liter×min on day 1, $p$<0.05). No appreciable differences in F response areas were detected in old animals (182,700±16,000 and 191,600±15,800 nmol/liter×min, respectively, on days 3 and 10 *vs.* 219,570±17,000 nmol/liter×min on day 1; $p$>0.05).

Hence, the F reaction to repeated stress exposure decreased in young animals on days 3 and 10 in comparison with immobilization 1. Old animals developed a less pronounced F reaction only on day 3.

Age-associated differences were also found for F values during different periods after the beginning of stress exposure on day 1: F concentrations in young animals were significantly higher 15, 30, 60, 240, and 1440 min after the beginning of exposure (Fig. 1). The F reaction area was lesser in old animals (219,570±17,000 *vs.* 272,730±16,000 nmol/liter×min in young animals, $p$<0.05). These data were in good agreement with our previous results indicating age-specific differences in F reaction to acute stress (2-h

immobilization) in the afternoon [5,7]. In addition, F concentrations after 30 and 1440 min on day 3 and after 30 min on day 10 (Fig. 1) were significantly lower in old animals than in young monkeys.

Comparative analysis of changes in the peripheral plasma DHEAS concentrations in monkeys of two age groups in response to repeated stress exposure showed the following shifts (Fig. 2). The concentrations of DHEAS in old animals were significantly lower than in young monkeys initially and during all periods of study in response to stress on days 1, 3, and 10 ($p<0.05$). These data were in good agreement with reduction of adrenal DHEAS secretion in aging primates [1,3,11].

The most marked elevation of DHEAS (similarly as of F) concentrations in young and old animals in response to stress was recorded on day 1 (Fig. 2). The maximum DHEAS levels were recorded in animals of both age groups after 120 min of exposure (191±10 and 299±40%, respectively). The levels remained high after 240 min and after 24 h.

The peak DHEAS concentrations (168±20%) in response to the third immobilization were recorded earlier (in 60 min) than on day 1 in young animals (Fig. 2). In old animals, DHEAS concentration peaked after 240 min and persisted at this level after 24 h (202±14 and 202±20%, respectively).

On day 10, DHEAS concentration in young animals reached the peak (142±7%) after 60 min and was significantly lower after 120 and 1440 min in comparison with the values in response to immobilization 1 (Fig. 2). No appreciable differences in DHEAS reactions to immobilization 10 and 3 were detected. Old animals did not develop an appreciable elevation of DHEAS concentration in response to immobilization procedure 10 in comparison with the initial level (Fig. 2). However, the initial DHEAS level on day 10 and DHEAS levels during all the tested periods after the beginning of exposure (15, 30, 60, 120, 240, 1440 min) were significantly higher than the initial DHEAS level on day 1.

Hence, the DHEAS reaction to stress (similarly as of F) decreased on days 3 and 10 in young animals. However, DHEAS elevation decreased less markedly than F elevation (in percent of initial level). As a result, the F/DHEAS molar concentration ratio in the peripheral blood in response to chronic stress did not change much (Table 1).

Old animals developed no appreciable reduction of DHEAS reaction to immobilizations 3 and 10. Moreover, a trend to an increase of basal DHEAS levels on day 3 and a significant increase of basal DHEAS levels on days 4, 10, and 11 in comparison with day 1 were observed. This resulted in a drastic (2-fold) drop of basal F/DHEAS ratio on days 3, 4, 10, and 11 and of its values 15, 30, 60, 120, and 240 min after the beginning of immobilization exposure 10 in comparison with the initial level on day 1 (Table 1).

Hence, old animals responded to repeated stress by a lasting elevation of DHEAS concentration and a pronounced decrease of F/DHEAS ratio. As DHEAS exhibited antiglucocorticoid characteristics [1,3,10,13], the F/DHEAS ratio was regarded as a better indicator of hypercortisolemia in humans and monkeys than measurements of blood F levels alone [3,8]. Reduction of F/DHEAS ratio as a result of repeated stress exposure indicated improvement of the hormonal status in old *Macaca mulatta*.

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