AGE DIFFERENCES IN THE HORMONAL RESPONSE OF THE ADRENALS AND TESTES TO STRESS IN MONKEYS

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Adrenal and testicular function of sexually immature and adult male baboons (Papio hamadryas) was investigated during immobilization stress. The concentrations of testosterone, 5α-dihydrotestosterone, hydrocortisone, and intermediate products of their biosynthesis – progesterone, pregnenolone, 17-hydroxyprogesterone, 17-hydroxypregnenolone, and 11-deoxycortisol – in the peripheral blood plasma of the monkeys were determined by a radioimmunological method. The main age differences in the character of the hormonal response to stress were shown to be the absence of changes in the blood androgen concentration of the immature animals and the smaller increase in the hydrocortisone concentration in them than in the adult monkeys. The concentrations of precursors of the steroid hormones fell considerably in the immature monkeys during stress, whereas in the adult animals their level rose 3-4-fold.

KEY WORDS: immobilization stress; steroid hormones; sacred baboons.

The important role of steroid hormones in adaptation to changing external environmental conditions is now generally accepted. However, there are few references in the literature to the study of the functions of steroid-secreting bands in sexually immature animals exposed to stress.

Some workers have demonstrated a great similarity between the endocrine systems in man and monkeys [1, 3]. This was the factor which determined the use of baboons (Papio hamadryas) as the experimental model.

The object of this investigation was a comparative study of the character of the hormonal response of the adrenals and testes in immature and adult monkeys during immobilization stress. The use of highly sensitive radioimmunological methods of determining steroids in small volumes of plasma in this study enabled the dynamics of concentrations not only of the end product of biosynthesis, but also of their principal precursors, to be recorded.

EXPERIMENTAL METHOD

The experimental animals were five immature (aged 3 years) and five adult male baboons born at Sukhumi Monkey Nursery. A stress situation was produced by immobilizing the unanesthetized monkeys strictly for 2 h on a special panel in the supine position. After 2 h the monkeys were returned to their customary situation. Blood for determination of the steroid concentration was taken from the cubital vein before and again 3, 6, 24, 48, and 72 h after immobilization. The concentrations of steroid hormones and their precursors in the blood plasma was determined by a radioimmunological method in a modification for monkey plasma [2]. Steroids were first isolated in the pure form by chromatography on Celite columns. The introduction of an internal standard into the plasma for extraction enabled the percentage of discovery of a given steroid to be determined in each sample, and, consequently, its absolute concentration could be calculated. The concentrations of the following steroids were determined in 1.2 ml plasma: testosterone, 5α-dihydrotestosterone, hydrocortisone (F), and the intermediate products – progesterone, pregnenolone, 17-hydroxyprogesterone, 17-hydroxypregnenolone, and 11-deoxycortisol.

Calculation of standard curves between logit-log coordinates, determination of the concentrations of the compound in the samples, and statistical analysis of the results (by Student's method) were carried out on the

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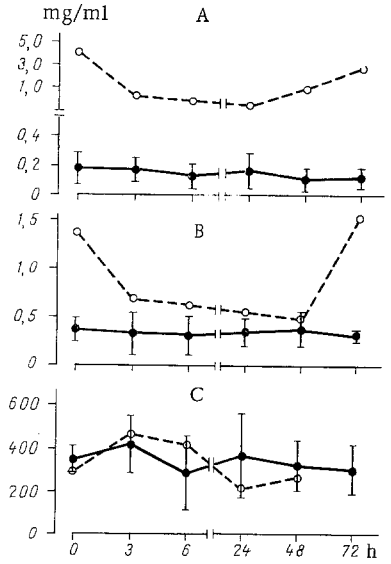

Fig. 1. Dynamics of concentration of testosterone (A), 5α-dihydrotestosterone (B), and hydrocortisone (C) in peripheral blood plasma of baboons after immobilization stress. Continuous lines with confidence limits indicate concentrations of compound in blood plasma of immature monkeys, broken lines – in blood of adult animals. Each point is arithmetic mean value for five monkeys. Abscissa, time of investigation (in h); ordinate, hormone concentration (in ng/ml).

TABLE 1. Individual Values of Concentrations of 11-Deoxycortisol and 17-Hydroxyprogesterone in Blood Plasma of Immature Baboons after Exposure to Stress

Steroids	Monkey no.	Before immobilization	Time after immobilization, h				
			3	6	24	48	72
11-deoxycortisol, mg/ml	14465	18,0	8,7	3,0	9,9	5,9	3,1
	14514	17,7	39,0	6,0	23,2	7,3	8,6
	14805	7,2	5,3	6,2	6,5	4,7	7,6
	14804	8,3	18,9	7,7	23,3	11,8	7,8
	14471	9,7	7,7	9,0	12,1	9,0	5,8
17-hydroxyprogesterone, pg/ml	14465	1136	386	194	322	153	144
	14514	1778	3595	328	1127	95	303
	14805	92	91	57	84	81	134
	14804	180	143	153	502	698	250
	14471	236	57	176	201	163	125

Soviet "Élektronika 15 VSM-5" computer by the use of specially devised programs [5].

EXPERIMENTAL RESULTS

The blood androgen concentrations of the 3-year-old baboons were virtually unchanged after exposure to stress, whereas in the adult males after immobilization for the same period of 2 h showed marked inhibition of testicular function, as manifested by a fall in the concentrations of testosterone and 5α-dihydrotestosterone; their concentrations remained low even two days after the end of immobilization (Fig. 1). However, it must be pointed out that the initial androgen level in the adult male baboons was many times higher than in the prepubertal males (testosterone 24 times, 5-dihydrotestosterone 6 times higher).

As Fig. 1 shows, 3 h after the beginning of immobilization the hydrocortisone concentration in the blood of the immature male baboons was increased by 20%, but it returned to its initial level after 6 h. In the adult males the hydrocortisone concentration during the first few hours after stress was increased by 70-80%, but was restored to normal again 24 h later.

Data on the dynamics of the concentration of 11-deoxycortisol, the immediate precursor of hydrocortisone, are given in Table 1. In two of five immature animals an increase in the concentration of this precursor was observed 3 h after immobilization, whereas in the other monkeys it was reduced.

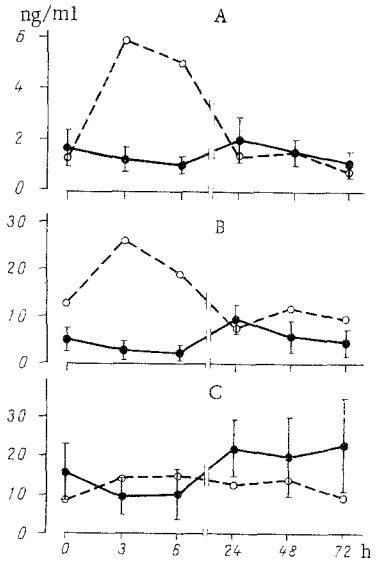

Fig. 2. Dynamics of concentrations of pregnenolone (A), 17-hydroxypregnenolone (B), and dehydroepiandrosterone (C) in peripheral blood plasma of baboons after immobilization stress. Remainder of legend as in Fig. 1.

Under conditions of stress, a noteworthy feature in the immature animals was that changes in the concentrations of steroids of the Δ^5-series were in the same direction (Fig. 2). A fall in their concentration was observed 3-6 h after the beginning of exposure to stress (the decrease was statistically significant for dehydroepiandrosterone and 17-hydroxypregnenolone; $P < 0.05$), but 24 h later this was replaced by an increase, although it was statistically significant only for 17-hydroxypregnenolone ($P < 0.05$). A different picture was observed in the adult male baboons: During the first few hours of exposure to stress the pregnenolone and 17-hydroxypregnenolone levels were increased 3-5-fold compared with initially, whereas the dehydroepiandrosterone concentration was unchanged. By contrast with the baboons of prepubertal age, in the adult males the pregnenolone and 17-hydroxypregnenolone concentrations in the blood returned to their initial level on the day after immobilization.

Despite considerable differences in the initial levels of 17-hydroxyprogesterone in the immature monkeys (Table 1), during the first few hours after exposure to stress its concentration mainly was reduced and remained low throughout the period of investigation. The blood 17-hydroxyprogesterone level in the adult males after immobilization increeased about fourfold (from 470 to 1680 pg/ml), and returned to normal after 24 h.

The blood progesterone concentration in the immature and adult monkeys was virtually unchanged following immobilization stress at between 300 and 400 pg/ml.

This investigation showed that the most significant age differences in the character of the hormonal response to stress are absence of a fall in the androgen concentration in the peripheral blood plasma of immature monkeys and a less marked increase in the hydrocortisone level compared with adult baboons. This last finding cannot be explained by the low sensitivity of the adrenal cortex of immature animals to pituitary ACTH, for after injection of ACTH into adult and immature monkeys the 17-hydroxycorticosteroid concentration in the latter is known to rise more than in adult animals [4]. Probably the quantitative differences in the hormonal response of monkeys of different ages to immobilization stress are due to the inadequate functional maturity of males of prepubertal age of certain adrenergic structures of the brain responsible for hypothalamic control over adenohypophyseal function. The absence of change in the androgen concentrations in immature males during stress may be explained both by the low sensitivity of the testes of prepubertal monkeys to gonadotropins and the low sensitivity of the adenohypophysis itself to hypothalamic releasing factors. This explanation is supported by the fact that after injection of releasing factor, stimulating LH secretion, in immature male monkeys no increase was found in the plasma testosterone concentration.

The considerable decrease in the concentrations of the two main precursors in the system of steroid hormone synthesis, namely pregnenolone and 17-hydroxypregnenolone, in monkeys of prepubertal age during the first few hours of exposure to stress can evidently be regarded as the result of their more intensive utilization for hydrocortisone synthesis in sexually immature animals compared with adults.

The marked individual scatter of the initial 17-hydroxyprogesterone concentration in immature male baboons is probably due to the fact that two of the experimental males (Nos. 14465 and 14514) were closer to the period of puberty, and that a hormonal response to stress began to be exhibited in them just as in adult animals as a result of simply being used in the experiment, although in their testosterone concentration they were indistinguishable from the other monkeys of prepubertal age. Considering that 24 h after immobilization no increase in the 17-hydroxyprogesterone level was found in the immature monkeys exposed to stress against the background of a raised 17-hydroxypregnenolone level, it can be postulated that the limiting stage in corticosteroid synthesis during stress is the conversion of 17-hydroxypregnenolone into 17-hydroxyprogesterone. Considering also that in the monkeys under conditions of stress an increase in the blood pregnenolone concentration but not of the progesterone concentration was observed, this suggests that hydrocortisone synthesis in these animals takes place predominantly along the pathway pregnenolone → 17-hydroxypregnenolone → 17-hydroxyprogesterone, but not along the pathway pregnenolone → progesterone → 17-hydroxyprogesterone.

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DETECTION OF ATYPICALLY LIGHT AND IMMUNOLOGICALLY DEFECTIVE ERYTHROCYTES IN PATIENTS WITH PAROXYSMAL NOCTURNAL HEMOGLOBINURIA

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Centrifugation of defibrinated blood on a density gradient at 600g for 30 min revealed the presence of atypically light erythrocytes, located on the boundary between plasma and Ficoll solution with a specific gravity of 1.077 g/cm^3 in 24 of 26 patients with paroxysmal nocturnal hemoglobinuria (PNH). Atypically light erythrocytes are characterized by a reduced content of rhesus-antigen and by a positive direct Coombs' test with anticomplementary serum. The appearance of atypically light erythrocytes in the upper layer was observed in patients with autoimmune hemolytic anemia and with hypoplastic anemia following splenectomy, and starting from the first day after the operation. It is suggested that the presence of atypically light erythrocytes is connected with a deficiency of the immune serum in patients with PNH and, primarily, with deficient ability of the spleen to eliminate defective erythrocytes.

KEY WORDS: paroxysmal nocturnal hemoglobinuria; density gradient.

The pathogenesis of paroxysmal nocturnal hemoglobinuria (PNH) has not yet been explained [3, 5]. The principal current hypothesis is based on the assumption that in this disease two erythrocyte populations – healthy and pathological – exist and are responsible for increased hemolysis in the blood stream [8-10].

The object of this investigation was to fractionate erythrocytes of patients with PNH by centrifugation on a density gradient and to study their immunologic properties.

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