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CIRCADIAN RHYTHMS OF BLOOD LEVELS OF CORTICOSTEROIDS AND THEIR PRECURSORS IN *Papio hamadryas* DEPENDING ON INITIAL FUNCTIONAL STATE OF THE HYPOPHYSEO-ADRENOCORTICAL SYSTEM

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According to much evidence in the literature adrenocortical function undergoes well-defined rhythmic changes in the course of the 24-h period [1, 2, 6-9]. However, most experimental studies of circadian rhythms of blood hormone levels have been carried out on small laboratory animals, which differ considerably from man in their biological parameters and in the spectrum of the steriods produced. Furthermore, technical difficulties until recently have prevented determination of blood levels of intermediate products of steroid biosynthesis, and this has greatly restricted the possibility of a systematic analysis of the functions of steroid-producing glands during the 24-h period. With the development and introduction of highly sensitive radioimmunologic methods, preceded by chromatographic fractionation of individual compounds, it is now possible to make a qualitative and quantitative assessment of a broad spectrum of steroids, including intermediate products of their synthesis.

Investigations have shown that monkeys and, in particular, the baboom *Papio hamadryas*, bear the closest resemblance to man in the character of steroid hormone production and metabolism [1, 2, 4]. This fact was the deciding factor in the choice of this species of baboon in order to study circadian rhythms of plasma steroid hormone levels.

The object of this investigation was to study the character of circadian rhythms of blood levels of steroid hormones (cortisol, aldosterone, and their precursors) in baboons and the dependence of blood steroid levels and manifestation of their circadian rhythms on the functional state of the hypothalamo-hypophyseo-adrenal system.

EXPERIMENTAL METHOD

Eighteen clinically healthy sexually mature male baboons aged 12-18 years and weighing 25-35 kg were used. The animals were divided into two groups. Group 1 consisted of intact baboons, kept together with a group of females without preliminary adaptation to short-term fixation and blood taking. Circadian rhythms of the blood steroid levels of the unadapted baboons were studied four times during the year at intervals of 3 months; the results were pooled. Animals of group 2 were adapted to the experimental conditions. For 15-30 days these baboons were kept in individual metabolic cages, and every day blood taking was simulated and accompanied by short-term fixation. Blood for steroid hormone assay was taken in its volume of 8-10 ml from the cubital vein into heparinized centrifuge tubes, at 3-hourly intervals for the 24. The first blood samples was taken at 9 a.m. and the last at 9 a.m. next day. Plasma was obtained by centrifuging the blood at 3000 rpm for 5 min and was kept in the frozen state in a refigerator at −20°C.

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TABLE 1. Mean Plasma Levels (in mg/ml) of Cortisol, Aldosterone, and Their Precursors in the 24-h Period during Adaptation of Baboons to Experimental Conditions (M ± m)

Steroid	Intact animals	Animals adapted for 15 days	P	Animals adapted for 30 days	P
Cortisol	349±26,00	308±43,00	<0,01	238±15,00	<0,01
Aldosterone	1,1±0,21	—		1,2±0,19	<0,10
11-deoxycortisol	9,7±0,62	8,1±0,65	<0,01	8,0±0,36	<0,01
17-hydroxypregnenolone	8,4±0,32	5,1±0,54	<0,02	4,4±0,41	<0,25
17-hydroxyprogesterone	0,5±0,04	0,2±0,01	<0,01	0,2±0,02	<0,10
Pregnenolone	0,8±0,29	1,1±0,20	<0,01	0,7±0,16	<0,01
Progesterone	0,7±0,03	0,5±0,02	<0,02	0,3±0,01	<0.20

The plasma cortisol level of the baboons was determined by the competitive binding with protein method [8], and levels of aldosterone, 11-deoxycortisol, progesterone, pregnenolone, 17-hydroxyprogesterone, and 17-hydroxypregnenolone levels were determined by a radioimmunologic method using highly specific antisera [2].

The standard curve was calculated, the steroid concentrations in the samples determined, and the results subjected to statistical analysis by Student's t-test on the 15-VCM-5 computer, using specially compiled programs.

EXPERIMENTAL RESULTS

The experimental results, illustrated in Figs. 1 and 2, showed that the blood levels of cortisol and all its precursors in intact male baboons vary considerably during the 24-h period; their concentration falls during the evening and night, and is maximal during the morning. The exception was 17-hydroxypregnenolone, the fluctuations of which during the 24-h period were small, and whose level rose in the course of the day in the manner of a stress reaction. The character of the curves reflecting changes in steroid levels during the 24-h period in baboons adapted to the experimental conditions was similar to that of curves for intact males for cortisol, 11-deoxycortisol, and pregnenolone only. The progesterone and 17-hydroxyprogesterone levels in this group of animals showed no characteristic cyclic changes, whereas the blood 17-hydroxpregnenolone level showed a clearly defined circadian rhythm. This change in the character of the circadian rhythms of steroid precursors may perhaps indicate a reorganization of steroid production depending on ecologic conditions and could indicate that cortisol synthesis via the pregnenolone-17-hydroxypregnenolone pathway is dominant in adapted baboons. In man, in a normal emotional and physical state, circadian fluctuations in the cortisol level are similar to those now discovered in adapted baboons [6, 10].

It is a noteworthy fact that the blood levels of cortisol and all its precursors in intact baboons at 9 a.m. the next day were significantly higher than at 9 a.m. on the first day. A different picture was observed in the adapted animals. In them there was no difference in the blood steroid levels at 9 a.m. on the first and second days of blood taking. The rise in the steroid levels at 9 a.m. on the second day in intact males must be regarded as a manifestation of stress due to the activating effect of the experimental conditions on the hypothalamo-hypophyseo-adrenal system. As a result the acrophase was shifted by 3 h for all steroids, leading to desynchronization of function of the hypophyseo-adrenal complex. Keeping the baboons in individual metabolic cages led to a significant fall in the absolute blood steroid levels (Table 1; Figs. 1 and 2).

The fall in the mean blood steroid level during the 24-h period was evidently due to keeping the baboons separately, for they normally lead a gregarious mode of life with a recognized hierarchy and also to the conditions of relative rest and limitation of motor activity. Similar results have been obtained in Professor N. A. Yudaev's laboratory [5] and by other workers in experiments on ordinary laboratory animals.

In the present investigation special attention was directed to changes in the blood aldosterone level during the 24-h period (Fig. 1). In animals which were unadapted and kept in large cages with a group of females the changes in the blood aldosterone level during the 24-h period were similar to those in the levels of cortisol and its precursors. After a 30-day period of adaptation the blood aldosterone level in the baboons during the morning fell by almost half, and subsequently during the 24-h period it increased statistically significantly with each successive taking of blood, to reach a maximum by 6 a.m. No cyclic fluctuations

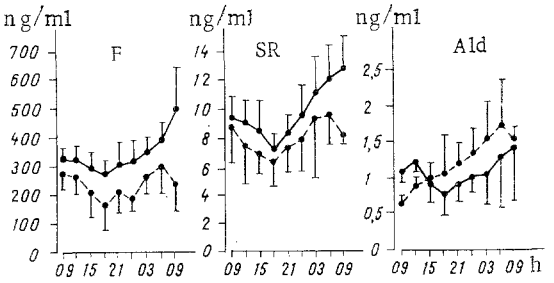

Fig. 1

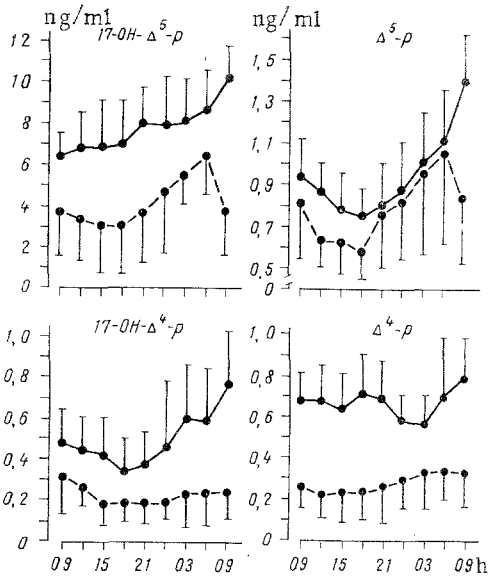

Fig. 2

Fig. 1. Changes in plasma levels (M ± m) of cortisol (F), 11-deoxycortisol (SR), and aldosterone (Ald) in baboons during the 24-h period. Here and in Fig. 2: continuous line — intact baboons; broken line — adapted baboons.

Fig. 2. Changes in plasma levels (M ± m) of pregnenolone (Δ^5 = P), progesterone (Δ^4 = P), 17-hydroxypregnenolone (17-OH-Δ^5-P), and 17-hydroxyprogesterone (17-OH-Δ^4P) in baboons during the 24-h period.

in the blood aldosterone level of these monkeys was found during the 24-h period, and its mean concentration for that period, by contrast with glucocorticoids (Table 1), did not fall during adaptation, because of the considerable rise in its level in the course of the 24-h period.

According to data in the literature [3] the dynamics of the aldosterone concentration during the 24-h period differed significantly in man when leading a normal mode of life and when engaged in intensive muscular activity.

The results thus indicate that changes in ecologic conditions under which animals are kept, limitation of their motor activity, and the presence of stress factors (repeated fixation of the animals and taking of blood samples) significantly change the orientation of steroid production and the absolute blood steroid levels, reflecting a change in the function of the hypothalamo-hypophyseo-adrenal system and also a circadian rhythm of the mineralocorticoid and glucocorticoid functions of the adrenals. Like man, *P. hamadryas* is characterized by a monophasic circadian rhythm of the blood steroid levels with a maximum in the early morning and a minimum in the evening.

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