Prolonged and repeated irradiation thus induces a lasting decrease in the ability of hematopoietic CFUs to maintain their own population. The disturbance of this unique property of CFUs is evidently linked with an irreversible decrease in the stem-cell pool of the hematopoietic system after irradiation.

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CIRCADIAN RHYTHMS OF SEX STEROIDS IN FEMALE BABOONS DURING PROLONGED HYPOKINESIA

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UDC 612.621.31-06:612.766.2]"52"

KEY WORDS: baboons (Papio hemadryas); steroid hormones; hypokinesia; circadian rhythms.

The widespread character of hypodynamia in modern society necessitates a comprehensive study of the effect of this extremal factor on women and on the reproduction system in particular. The most adequate model for research of this kind is provided by female baboons (Papio hemadryas), in which the basic parameters of activity of the hypothalamic–hypophyseal–gonads system are very close to those in man [1, 3]. Since the circadian rhythm of secretory activity of the gonads is one of the most important characteristics of functional integrity of the hypothalamic–hypophyseal–ovarian system [8], experiments were carried out on female baboons in order to study circadian rhythms of hormonal activity of their ovaries and adrenals during prolonged clinostatic hypokinesia. This paper describes the results of a comparative study of circadian rhythms of the estradiol, progesterone, testosterone, and cortisol levels in the peripheral blood plasma of unrestrained female baboons and of baboons whose movements were restricted, in different phases of the menstrual cycle.

EXPERIMENTAL METHOD

Experiments were carried out on 10 mature fertile female baboons weighing 12-16 kg, aged 5-10 years, with a stable biphasic menstrual cycle lasting 28-35 days. The cycles were monitored by noting swelling of the "genital skin," which is the target tissue for estrogens and reflects proliferative processes developing in the reproductive system in the follicular phase of the cycle. To study circadian rhythms of the steroid levels (control) blood samples were taken from monkeys unrestrained in their cages, with natural alternation of daylight and darkness, every 6 h for the 24-h period starting from 12 noon in the follicular (6th-8th day) and luteal (6th-

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Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 98, No. 9, pp. 348-351, September, 1984. Original article submitted July 26, 1983.

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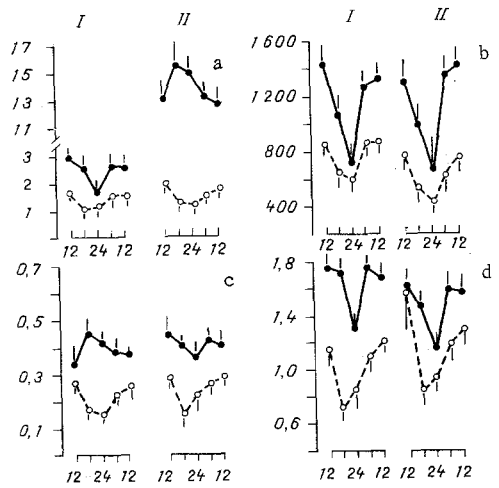

Fig. 1. Circadian rhythm of sex steroid and cortisol levels in plasma of female baboons (P. hemadryas) with unrestrained (filled circles) or restricted (empty circles) motor activity. a) Progesterone, b) cortisol, c) estradiol, d) testosterone. I) Follicular phase, II) luteal phase of cycle. Abscissa, time of day (12 = noon, 24 = midnight); ordinate, plasma steroid concentration (in mM).

7th day after ovulation) phases of the cycle. Circadian rhythms during hypokinesia were studied in animals of the same group on the 15th day of restricted movement. Five baboons were immobilized in the follicular (6th-7th day) and five in the luteal (6th-7th day after ovulation) phases of the cycle. The monkeys were fixed in the horizontal position by the clinostatic hypokinesia method [4]. Individually made immobilization suits, fixed on special supports, enabled hind limb movements to be completely and head movements partially prevented for a long time, while allowing the fore limbs to move freely. Blood samples were taken at noon, 6 p.m., midnight, 6 a.m., and noon, i.e., at times corresponding to those in the control experiment. Blood was taken from the cubital vein and plasma was separated by centrifugation, then frozen and kept at 20°C until required for steroid determination.

The hormonal function of the gonads was estimated by determining plasma levels of the following steroid hormones: estradiol, progesterone, and testosterone. The concentration of these hormones was determined by radioimmunoassay adapted for monkey plasma [2], using highly specific antisera. The cortisol concentration was determined by the competitive binding method [7], enabling assessment of the adrenal glucocorticoid function. The results were subjected to statistical analysis by computer using Student's test.

EXPERIMENTAL RESULTS

It will be clear from Fig. 1b that the circadian rhythm of the plasma cortisol level of the baboons was characterized by a gradual fall in the steroid concentration in the course of the 24-h period. The minimal values were recorded at midnight, and later the hormone level rose to reach a peak during the morning (from 6 a.m. to 12 noon). The character of the circadian rhythm was similar in both phases of the menstrual cycle. This rhythm of the cortisol level in female baboons is in agreement with data in the literature [3, 6]. It is also similar to the character of the circadian rhythm of cortisol in women [5].

Circadian fluctuations in the testosterone level (Fig. 1d) were similar in character and correlated highly with the cortisol rhythm in both phases of the cycle ($r = 0.976$ in the luteal and $r = 0.821$ in the follicular phase). The mean concentrations of these two hormones for the 24-h period (Fig. 2b, d) had a tendency to decrease in the luteal phase of the cycle, but the amplitude of the circadian fluctuations of both steroids was unchanged in the course of the cycle.

In the follicular phase of the cycle circadian fluctuations in the progesterone level correlated highly with the circadian rhythm of cortisol ($r = 0.869$; Fig. 1a), suggesting that the adrenals participate in formation of the circadian rhythm of progesterone in the first phase of the cycle. In the luteal phase, however, when the concentration of the steroid was increased by more than 10 times and when the corpus luteum becomes the principal source of its biosynthesis, an "ovarian type" of circadian rhythm is established. Under these circumstances maximal values for the hormone were recorded between 6 p.m. and midnight, and minimal values in the afternoon. Meanwhile a tendency was observed for the amplitude of the circadian fluctuations in the level of the hormone to fall. A similar type of circadian rhythm of progesterone was found in female macaques [9] in the luteal phase of the cycle.

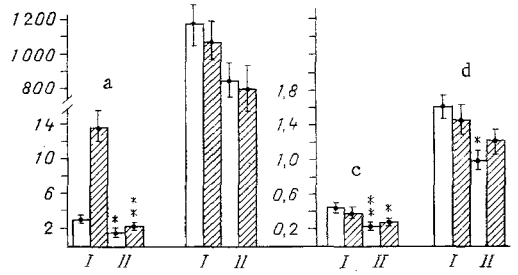

Fig. 2. Mean 24-hourly concentrations of sex steroids and cortisol in blood plasma of female baboons with unrestrained (I) and restrained (II) motor activity, in folicular (unshaded columns) and luteal (shaded columns) phases of cycle. *P < 0.05, **P < 0.001 compared with control. Remainder of legend as to Fig. 1.

Circadian fluctuations in the estradiol level in the follicular phase were characterized by a definite rhythm (Fig. 1c) with the maximum for the 24-h period between 6 p.m. and midnight, which is out of phase with the circadian rhythm of progesterone ($r = -0.481$). In the luteal phase of the cycle the trend of the circadian rhythm of estradiol was modified. Maximal concentrations were recorded in the afternoon and minimal at midnight. The mean 24-hourly concentration of the hormone under these circumstances was practically indistinguishable from values found in the early follicular phase of the cycle (Fig. 2c).

In view of the considerable individual variations in the steroid levels of the monkeys, the data in Table 1 are expressed as percentages of deviations from the mean 24-hourly concentration. As Table 1 shows, circadian fluctuation in cortisol and testosterone levels in both phases of the cycle, and in the progesterone and estradiol levels in the follicular phase of the cycle were significant ($0.001 < P < 0.02$).

The circadian rhythm of the cortisol and sex steroid levels in the peripheral blood plasma of the baboons on the 15th day of hypokinesia, shown in Fig. 1, and the lowest values between 6 p.m. and midnight and highest in the morning (from 6 a.m. to noon). The data in Table 1 indicate that these fluctuations were significant for all steroids studied ($0.01 < P < 0.05$). The coefficient of correlation for cortisol, on the one hand, and for testosterone, estradiol, and progesterone, on the other hand, was 0.840, 0.904, and 0.871 respectively for females immobilized in the follicular phase, and 0.904, 0.890, and 0.910 for baboons immobilized in the luteal phase.

Restriction of motor activity did not alter the general character of the cortisol and testosterone rhythm, although their circadian fluctuations took place against the background of a lower blood level of these steroids.

The change in parameters of the estradiol rhythm in the two phases of the cycle was highly significant during hypokinesia. The character of the circadian rhythm of the hormone in the follicular phase (Fig. 1c) was out of phase with its natural circadian rhythm in intact baboons. The amplitude of fluctuations of the steroid level was increased. In the luteal phase an increase in amplitude of fluctuations of the estradiol level led to the appearance of a distinct circadian rhythm, which was absent in intact animals in the luteal phase. Simultaneously with it, a progesterone rhythm in the same direction appeared (Fig. 1a). The high degree of correlation between the circadian changes in the levels of these two steroids with each other and with the cortisol and testosterone rhythms indicates an adrenal origin for their circadian rhythms in the follicular and luteal phases during hypokinesia.

As Fig. 2 shows, during hypokinesia there was a progressive decline in the mean 24-hourly concentrations of all steroids studied in the peripheral blood plasma. The level of decline of estradiol and progesterone was statistically significant in both phases of the menstrual cycle, whereas that of testosterone was significant only in the follicular phase. The mean 24-hourly cortisol concentration remained virtually unchanged in all animals exposed to hypokinesia.

The marked tendency for the amplitude of the cortisol rhythm to diminish in the luteal and follicular phases of hypokinesia in the presence of a parallel increase in amplitude of circadian fluctuations in the estradiol and testosterone levels must be noted.

TABLE 1. Amplitude of Circadian Fluctuations of Sex Steroid and Cortisol Levels with Unrestrained and Restricted Motor Activity (in % of deviation from mean 24-hourly concentration; M ± m)

Steroid	Phase of cycle	Control				P	Hypokinesia				
		period of maximal concentration		period of minimal concentration			period of maximal concentration		period of minimal concentration		
		12 noon	18 p.m.	24 midnight	12 noon		6a.m	12noon	24 midnight	18 p.m.	P
Estradiol	Follicular	—	110±7,6	—	82±4,8	<0,02	—	118±6,8	—	75±6,6	<0,01
	Luteal	105±4,3	—	87±3,0	—	<0,1	—	114±1,9	—	79±6,1	<0,01
Progesterone	Follicular	122±6,5	—	70±7,7	—	<0,001	—	123±12,5	—	70±15,0	<0,05
	Luteal	—	122±13,7	—	92±5,6	<0,1	—	121±5,0	—	80±3,4	<0,01
Testosterone	Follicular	107±3,0	—	79±5,3	—	<0,001	—	124±1,7	—	73±10,0	<0,01
	Luteal	109±11,3	—	72±8,2	—	<0,02	—	139±4,8	—	80±5,4	<0,01
Cortisol	Follicular	126±7,3	—	61±4,3	—	<0,001	—	115±7,7	—	74±17	<0,05
	Luteal	121±6,4	—	59±5,8	—	<0,001	—	122±9,5	—	70±1,3	<0,01

The results of this investigation thus demonstrate internal desynchronization of circadian rhythms of the sex steroid levels during prolonged restriction of motor activity of female baboons irrespective of the phase of the cycle during immobilization, and which is manifested as a change in the position of the acrophases, a change in the amplitude of the rhythm, and a decrease in the mean 24-hourly concentrations. However, the mechanisms lying at their basis still remain largely unexplained.

It can be tentatively suggested that blocking of the hormonal activity of the developing follicle and corpus luteum weakens the role of the ovaries in the formation of the peripheral pool of progesterone and estradiol drastically, and the leading role in the process now switches to the adrenals. This leads to disappearance of the "ovarian type" of circadian fluctuations in the estradiol and progesterone levels recorded for estradiol in the period of maturation of the dominant follicle, and for progesterone in the period of maximal function of the corpus luteum.

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