Infradian Fluctuations in Serum Testosterone Levels in Male Laboratory Rats

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 151, No. 5, pp. 577-580, May, 2011
Original article submitted February 3, 2010

The dynamics of serum testosterone was studied in laboratory male rats kept separately from females. Infradian rhythm of changes in testosterone level equal to 4 days and ultradian (within 24 h) periods equal to 160 and 480 min were detected. The maximum serum level of testosterone in male rats was synchronized with the greatest number of estrous females.

Key Words: *testosterone; infradian rhythm; rat; estrous cycle*

Serum testosterone level in laboratory rats is changing throughout 24 h and is the maximum during the evening and night hours [5]. Many-day (infradian) rhythms of blood testosterone levels in rodents are little studied.

A 4-day periodicity is normally characteristic of the estrous cycles of female laboratory rats [1]. The estrous cycles of rodents are synchronized when they are kept together [6]. Synchronization of sexual activity in rodents is biologically justified. Estrus females are the most vulnerable for predators because of changes in their behavior, and the loss of population members can be minimized by synchronization of this status in many individuals. In addition, synchronization of the male/female sexual activity ensures the maximum probability of copulation. Presumably, male rodents synchronize their reproductive activity with representatives of the opposite gender even under conditions of isolation.

However, we failed to find data on infradian fluctuations of testosterone levels in the blood of male rats. The 4-day periodicity of fluctuations of blood leukocyte absolute counts in male rats and 6-7-day periodicity of fluctuations of cell counts in the lymphoid organs of male laboratory mice prove the presence of infradian rhythms in these animals [4]. Infradian fluctuations of these parameters are presumably caused by the hormonal (including testosterone) effects on the immune system. According to some data [8], androgens inhibit the Th2 immune response and are characterized by anti-inflammatory effect.

Infradian rhythms of blood testosterone concentrations should be taken into consideration when measuring the individual levels of the hormone in laboratory rats and in studies of the neuroimmunoendocrine interactions.

We studied the rhythms of serum testosterone level fluctuations in laboratory rats.

MATERIALS AND METHODS

The study was carried out on adult Wistar males (n=8) and females (n=10) and outbred laboratory males (n=15) and females (n=32) from Stolbovaya Breeding Center. The body weight of males was 190-230 g. The animals were kept 2-3 per cage at natural light and 20°C. The males were kept separately from females in different buildings. The blood was collected from the tail vein. The sera were kept no longer than 2 weeks at -40°C. Estrous cycle phases were determined at 16.00-18.00 by colpocytogram. Serum testosterone was measured using ELISA kits (DRG) on an ANTOS microplate EIA analyzer. Testosterone level was evaluated in one test in each experimental series. The significance of differences between the parameters was evaluated by Mann–Whitney U test.

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RESULTS

In experimental series I, the levels of testosterone were measured in Wistar rats in blood specimens collected at 10.05-10.20 daily from January 28 to February 6, 2009. The maximum levels of serum testosterone were recorded in all animals of this group on January 28, February 1 and 5 (Fig. 1, *a*). However, the maximum levels of testosterone varied in different animals. For more demonstrative presentation of these results, the animals were divided into 2 groups: with more pronounced (more than 30%; group 1; n=5) and less pronounced (below 30%; group 2; n=3) shifts in testosterone levels. Comparative analysis of testosterone levels on January 29, February 2 and 6 showed no differences between these groups (p>0.05), while on January 28, February 1 and 5 the differences were significant (p≤0.01). The period between the peaks of blood testosterone levels in Wistar males was 4 days.

For more ample understanding of the dynamics of testosterone throughout 24 h, we carried out a series of experiments in which blood was collected from two outbred male laboratory rats every 80 min from 21.20 on July, 15 till 01.20 on July 17, 2009 (Fig. 2). Serum testosterone increased throughout 24 h every 160 min from midnight (local natural time), the peaks of testosterone being recorded with a period of 480 min at 00.00, 08.00, and 16.00. Importantly that the changes were synchronous in both rats (r=0.56), this indicating a regular nature of these changes. Previous studies [3] of motor activity of one-year-old outbred rats revealed the duration of the maximum activity: 160.05±0.07 min. Importantly that a period of 160 min was also detected in the geomagnetic activity spectrum [2].

For more accurate measurements of testosterone levels during the period of its maximum concentrations in the serum, blood was collected 3 times: 80 min before the presumable maximum, during expected peak, and 80 min after it. Hence, a 4-day rhythm of maximum testosterone levels was revealed in outbred male rats (n=6) on September 7-16, 2009, with the peaks recorded at 16.00 on September 8, 12, and 16, 2009 (Fig. 1, *b*).

Importantly that Ap index, reflecting the level of geomagnetic activity, did not correlate with fluctuations of serum testosterone levels (r<0.2 in all series of experiments).

The maximum testosterone levels in males in the evening corresponded to the day on which the maximum number of females was in the estrous phase. On August 23 and 27, 2009, the maximum levels of testosterone in the males (n=5) were detected at 8.00 and 16.00, the numbers of estrus females being the maximum (n=32) during the same hours (Fig. 3). Importantly that the males were isolated from the females and that manipulations on the males and females were performed by different workers not contacting during the experiments. The fact of synchronization of sexual activity of animals of different genders kept separately can be explained only by the existence of some other heretofore unknown external synchronizer.

Our data on the ultradian rhythm of testosterone fluctuations in laboratory rat males are in line with published data. The three-peak circadian testosterone curves with maximums at 02.00, 12.00, and 18.00 were demonstrated [12]. It has been found that testosterone is released into the circulation by pulses at 3-6-h intervals [9]. Other studies carried out exclu-

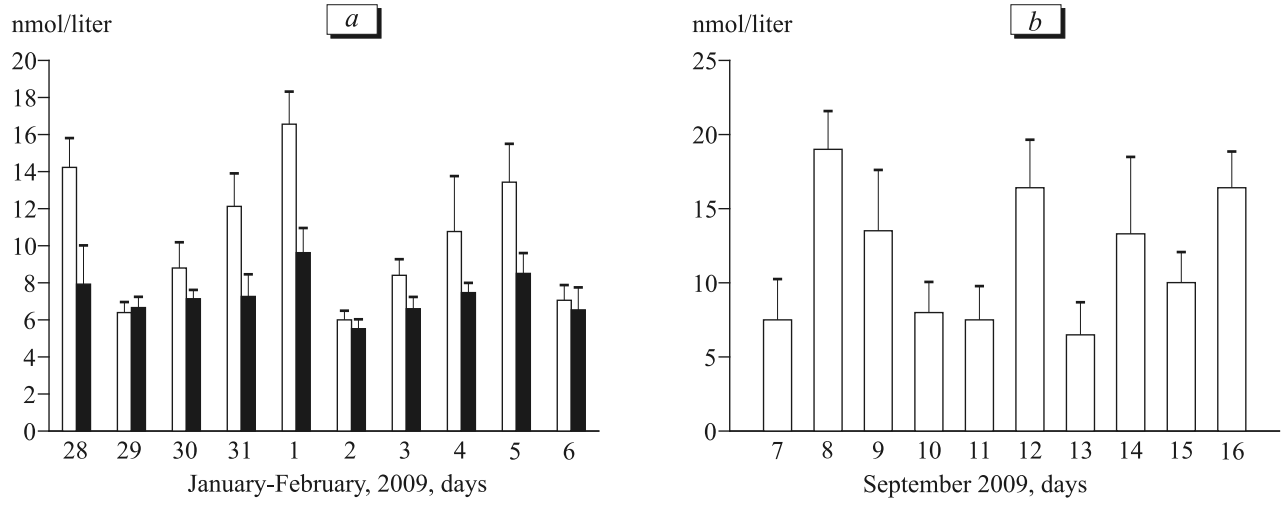

Fig. 1. Dynamics of serum testosterone levels in Wistar males (*a*) of group 1 (light bars) with pronounced fluctuations in the parameter and group 2 (dark bars) with slight changes in testosterone level from January 28 till February 6, 2009 (blood collected at 10.05-10.20 local natural time, Moscow), and in outbred male rats (*b*) at 16.00 local natural time (blood collected 80 min before expected peak, during expected peak, and 80 min after it) in September 2009.

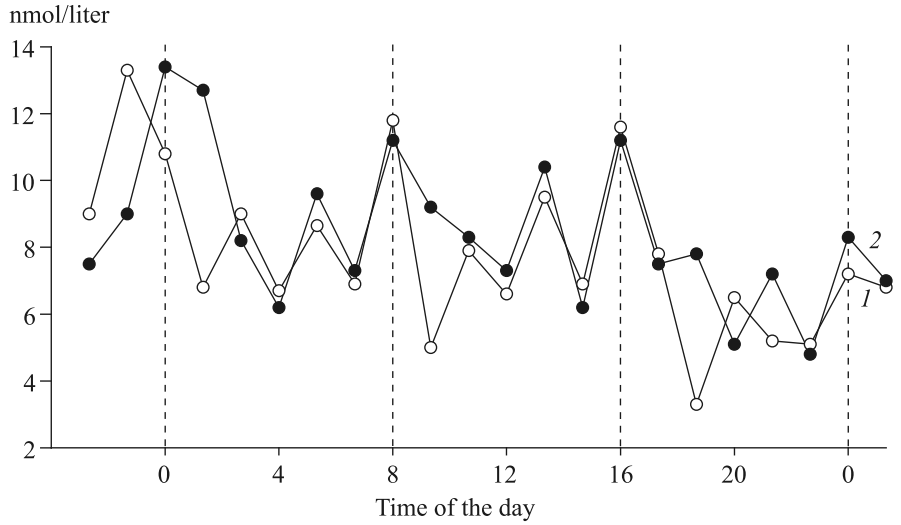

Fig. 2. Dynamics of blood testosterone levels in two outbred male rats (blood collected every 80 min from 21.20 on July 15 till 01.20 on July 17, 2009, local natural time, Moscow).

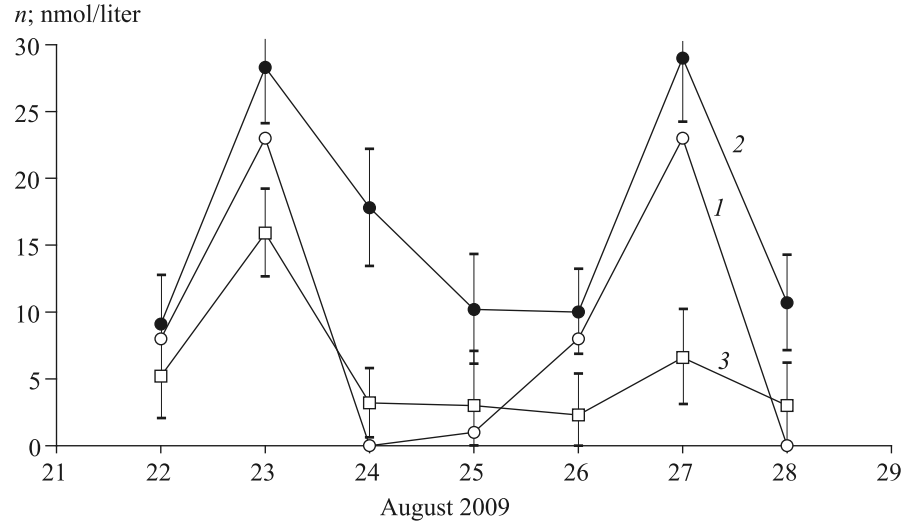

Fig. 3. Correlation between the numbers of outbred estrus females and serum testosterone levels in males (blood collected at 08.00 and 16.00 local natural time, Moscow), the males and females being kept at different laboratories. *1*) number of estrus females; *2*) testosterone level in males at 16.00; *3*) testosterone level in males at 08.00.

sively during the working day (from 7.00 to 16.00) recorded just the morning peak of testosterone at 08.00-10.00 [10,11,13].

Infradian fluctuations of testosterone have not been described, but a period of blood testosterone changes about a month long has been found in men, corresponding to the mean ovariomenstrual cycle in women [7]. Hence, our results confirm a similar regularity in laboratory rats.

Hence, serum testosterone level in male laboratory rats varies in accordance with the circadian rhythm and has ultradian periods of 160 and 480 min, their amplitudes increasing with prolongation of the period, and a many-day (infradian) period of 4 days. This fact should be taken into consideration in comparative studies of individual values characterizing the status of the nervous, immune, and endocrine systems.

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