Effect of Pinealectomy on Circadian Rhythm of Spermatogenesis

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The mitotic index of spermatogonia and 24-h dynamics of stages IV, VI, and XIV of spermatogenic cycle are characterized by circadian rhythm. No circadian rhythm was detected for 11 of 14 stages. Pinealectomy led to an increase of the mitotic index of spermatogonia but did not modulate the incidence of spermatogenic cycle stages, and led to disappearance of the circadian rhythm of both the mitotic index and spermatogenic cycle stages.

Key Words: *spermatogenesis; mitotic index; circadian rhythm; epiphysectomy*

The duration of spermatogenesis in mammals is very long (in albino rats this process lasts for 48 days), which suggests the possibility of its circadian time dynamics. The synthesis of DNA in spermatogonia of mice is characterized by a circadian rhythm [6]. Spermatogenesis includes 4 phases, each consisting of certain cell types. All phases are intrinsic for any site of the seminal tubule, the course of these phases determining the regular alternation of cell compositions. Based on this, a notion on the spermatogenic cycle is formulated, according to which the cycle in albino rats has 14 stages and lasts for 12 days [9]. The stages are situated in succession along the seminal tubule, one following the other, thus forming the wave of spermatogenic layer. Hence, spermatogenesis is a complex periodical process, which can be represented as an hierarchy of biorhythms of lesser duration.

The 24-h dynamics of spermatogenesis and its possible regulation were not studied. We investigated the 24-h dynamics of spermatogenic cycle stages, mitotic index (MI) of spermatogonia, and effects of pinealectomy on them.

MATERIALS AND METHODS

Experiments were carried out on male albino rats kept under standard day/night regimen (illumination from 6.00 to 18.00). On day 40 after pinealectomy the animals were sacrificed every 3 h over 2 days, and thus the process was followed during 2 periods of circadian rhythm. In order to evaluate the incidence of spermatogenic cycle stages, 300 seminal tubules were examined in each animal. The duration of stages was estimated by the formula:

$$t=(T/k)\times N,$$

where t is duration of the stage in hours, T duration of spermatogenic cycle in hours, k number of studied objects, and N incidence of stages.

The spermatogonia MI was determined in tubules at the spermatogenic cycle stage VI, as dividing spermatogonia are most incident during this period. A total of 1000 spermatogonia from each animal were examined in order to determine MI. The periodicity of processes was evaluated using spectral analysis [1], by comparing the values at night and in the daytime, and by the pattern of smoothed curve plotted using the least squares method.

RESULTS

The incidence of stages of the spermatogenic cycle is inversely proportional to the stage duration. By their incidence, the 14 stages of spermatogenic cycle can be divided into 3 groups. Group 1 includes stages I, VII, and VIII with 100-190‰ frequency and duration of

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30-55 h; group 2 includes stages II, III, IV, V, XII, XIII, and XIV with 50-75‰ frequency and duration of 14-20 h; and group 3 includes stages VI, IX, X, and XI with 30-40‰ frequency and duration of 9-14 h (Table 1). The longest stages are those reflecting the period of formation. Group 2 stages characterize the differentiation of spermatogonia and period of maturation; the final stages reflect type B spermatogonia division and leptotene.

Spectral analysis showed circadian rhythm of the 24-h dynamics for stages IV, VI, XIII, and XIV. However the differences in the dark and light hours were observed only for the first 3 stages. During stages VII, VIII, and XII the night and day frequencies were different, but spectral analysis showed no circadian rhythm. Smoothed curve presenting the frequencies of stages IV, VI, and XIV was sinusoidal, while for the rest cases it was either a straight line or a sinusoid with slight amplitude.

The mean 24-h MI of spermatogonia was 118.18±1.35‰. Circadian rhythm of MI (Fig. 1, *a*) with acrophase during the first hours of the light period was observed. The daytime MI was significantly higher than the night MI value (Fig. 1, *b*). Spectral analysis showed an ultradian rhythm with 8-9-h period.

Pinealectomy did not change the mean 24-h incidence of the stages (Table 1), but the circadian 24-h dynamics of incidence was lost for stages IV, VI, and XIV. The incidence in the night and day hours did not differ appreciably for all stages, and the smoothed curves acquired the sinusoidal shape with a poorly expressed amplitude.

The mean MI of spermatogonia after pinealectomy increased significantly from 118.18±1.35 (control) to 139.45±1.35‰ (epiphysectomied animals, $p<0.01$); the ultradian rhythm did not change, while the circadian rhythm of spermatogonia MI disappeared (Fig. 1, *a*).

Leblond and Clermont introduced the notion of a spermatogenic cycle into science [9]; the length of the cycle along the seminal tubule was called spermatogenic wave. These waves follow each other along the convoluted tubule; this led to the formation of a concept of wave-like spatial organization of spermatogenic tissue [2] (spermatogenesis as a combination of different rhythms). On the one hand, spermatogenesis is alternation of periods of multiplication, growth, maturing, and formation; on the other, all these processes are involved in the spermatogenic cycle and spermatogenic wave. Hence, spermatogenic cycle and wave are subjected to regulatory effects. Spermatogenesis depends on the photoperiod in all animals; therefore it can be expected that the 24-h dynamics of spermatogenesis, spermatogenic cycle, and wave depends on the duration of photoperiod. However circadian rhythms were detected only for 3 of 14 stages (IV, VI, and XIV). On the other hand, cell types characteristic of certain periods of spermatogenesis were observed at different stages. Unification of the incidence of stages, reflecting individual processes of spermatogenesis, showed a circadian rhythm for the group. This rhythm was detected when we united stages II-IV, IV-VI, and meiosis stages XII-XIII including the zygotene of reduction division. Moreover, if any spermatogenesis process started and ended during

TABLE 1. Incidence of Stages (‰) of Spermatogenic Cycle (*M*±*m*)

Stage	Intact animals			Epiphysectomied animals		
	mean for 24 h	night hours	daytime	mean for 24 h	night hours	daytime
I	105.14±2.14	104.00±2.46	107.83±6.09	104.66±3.16	105.93±6.36	101.93±4.83
II	48.90±1.06	48.82±2.03	49.78±0.76	47.31±1.86	43.96±2.93	46.02±1.33
III	74.52±2.13	73.03±2.83	80.38±3.36	77.78±1.86	74.69±3.90	79.09±1.57
IV	59.28±1.92	56.31±3.30*	66.03±2.26	56.14±2.52	57.28±3.00	51.62±4.59
V	57.46±1.71	59.21±3.50	59.27±2.70	62.85±2.26	63.1±1.0	57.71±3.93
VI	32.77±1.45	31.90±1.46*	34.56±2.70	37.34±1.45	38.93±2.43	34.63±1.37
VII	187.96±3.58	181.15±4.13*	198.04±5.19	183.39±4.87	181.58±7.89	193.51±6.23
VIII	132.38±4.42	136.50±6.09*	118.81±4.33	129.63±2.90	122.31±5.39	136.06±3.56
IX	40.74±1.89	40.73±3.36	37.40±2.56	37.62±1.98	41.06±2.60	39.93±3.06
X	30.27±1.13	29.40±2.36	31.37±1.67	27.37±1.02	26.04±1.50	29.27±3.83
XI	36.74±1.56	39.26±2.43	36.13±1.96	38.04±2.15	35.06±2.43	38.33±3.00
XII	71.26±2.32	75.62±3.36*	65.20±2.33	73.98±2.22	80.12±2.70	68.36±3.50
XIII	65.02±1.92	64.6±2.4	67.9±4.4	62.50±2.14	60.64±3.13	58.61±3.56
XIV	55.29±2.43	62.04±2.93*	47.45±3.53	52.54±2.55	56.48±5.03	53.08±2.89

Note. $p<0.01$compared to daytime value.

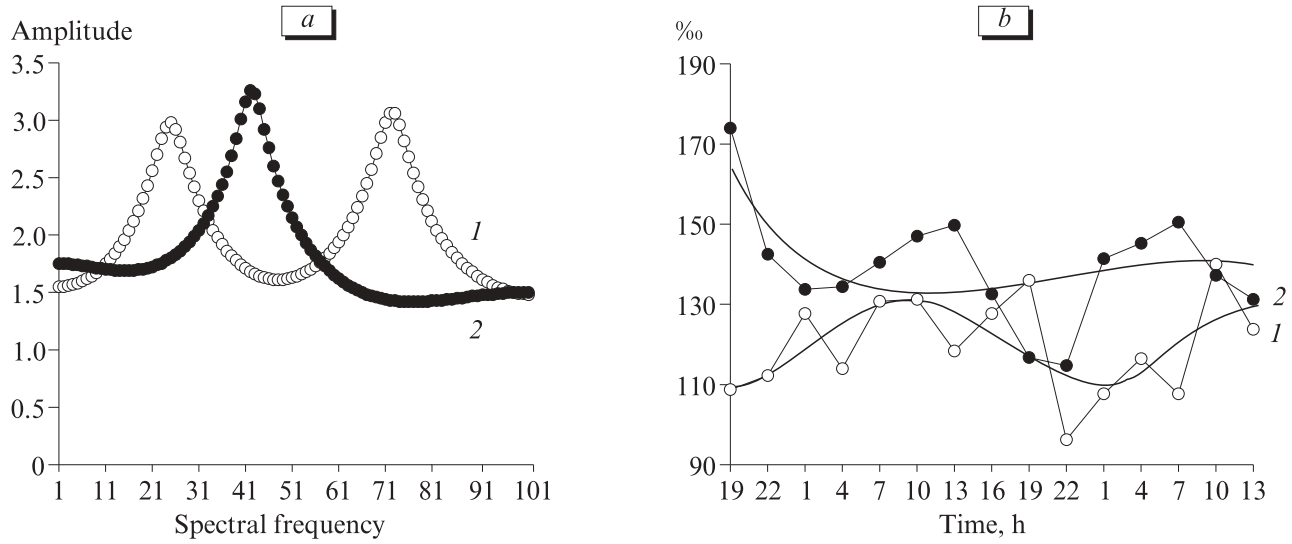

Fig. 1. Mitotic index of spermatogonia. *a*) spectral analysis; *b*) 24-h dynamics and smoothed curves; *1*) control animals; *2*) epiphysectomied animals.

one stage of the cycle, the course of this stage exhibited a circadian rhythm: stages IV (multiplication of the intermediate spermatogonia), VI (multiplication of type B spermatogonia), and XIV (reduction and equation meiosis division).

Since hypophysectomy, injections of testosterone, LH, FSH do not modify the spermatogenic cycle and wave [4,5,7], presumably, cell combinations, and, hence, spermatogenic cycle and wave emerge because of simultaneous course of all periods of spermatogenesis at any portion of the seminal tubule; this course is characterized by justified photoperiodical dependence. The latter fact is confirmed by circadian rhythm of spermatogonium MI, its disappearance after epiphysectomy, circadian rhythm of incidence of spermatogenic cycle stages IV, VI, and XIV, and groups of stages reflecting individual spermatogenesis processes. This fact also attests to the role of the pineal gland in the formation of spermatogenesis circadian rhythm. In addition, not all animal species have spermatogenic cycle and wave [8]. It seems that spermatogenic cycle and wave are manifestations of spatial organization of spermatogenic tissue, while spermatogenesis is a process with circadian rhythm regulated by the pineal gland.

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