EXPERIMENTAL BIOLOGY

Effects of Pineal Peptides on Circadian Dynamics of Spermatogonia Proliferation in Albino Rats

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Mitotic index of type B spermatogonia in intact animals is characterized by a circadian rhythm. Mitotic index increased and its circadian rhythm disappeared after pinealectomy. Treatment with Epithalamin for 14 days restored the circadian rhythm. The circadian biorhythm of spermatogonia proliferation suggests the presence of circadian rhythm of spermatogenesis in general and its regulation by the pineal gland.

Key Words: *spermatogenesis; pinealectomy; pineal peptides; circadian rhythm*

Reproductive function of monoestrous animals is characterized by seasonal rhythms formed with participation of the pineal gland. The dependence of polyestric animals reproduction on the season is less pronounced. Apart from the seasonal rhythm, the reproductive function is characterized by a circadian rhythm, which depends on the rhythm of motor activity and photoperiod. Production of gametes is a components of the reproductive function. The duration of spermatogenesis in animals varies, but in general it is several tens of days. The presence of circadian changes during this period can be hypothesized.

The pineal gland plays an important role in the formation of circadian periodicity [4]. Proliferation in tissues (epithelial, lymphoid) is characterized by a circadian rhythm, which is regulated by the pineal gland [2,3]. It seems that circadian rhythm of proliferation is characteristic of all tissues.

The involvement of the pineal gland in the regulation of proliferation biorhythm is beyond doubt, but the mechanism of this regulation remains unknown. The pineal gland produces melatonin and some bioactive oligopeptides, whose functional role is now studied. The process of spermatogenesis starts with multiplication of spermatogonia, circadian dynamics and regulation of this process remain unknown.

We studied circadian dynamics of spermatogonia proliferation and effects of the pineal gland and pineal peptides on this process.

MATERIALS AND METHODS

Experiments were carried out on 270 male albino rats (180-200 g) kept under 12/12h day/night regimen (day at 6.00-18.00). The animals were adapted to illumination regimen for 2 weeks and then divided into 3 groups: control (intact), pinealectomy, and pinealectomy+Epithalamin treatment. Pinealectomy was carried out under general thiopental narcosis. Epithalamin (a complex of pineal oligopeptides) was injected for 14 days until sacrifice (once a day after onset of the dark period). On day 40 after pinealectomy the animals were sacrificed by ether narcosis every 3 h for 48 h. Circadian dynamics of the mitotic index (MI) of type B spermatogonia was studied at stage VI of spermatogenesis cycle, during which their division is most intensive. Cyclic pattern of the processes was determined by the method of spectral analysis, comparison of the mean MI values during the dark and light period, and analysis of smoothed curve reflecting circadian changes in MI and plotted using the method of

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least squares. The results were statistically processed using Fisher—Student's test [1].

RESULTS

Spermatogenesis takes place in convoluted seminal tubules. Type B spermatogonia observed during stages V and VI of spermatogenic cycle are located near the tubular basal membrane and have oval nuclei with appreciable number of compact chromatin granules. In the nuclei chromatin granules form an uneven crust near the membrane. The nucleoli of different shape are usually not connected to the karyolemma. Division of type B spermatogonia at stage VI of spermatogenic cycle is the last of spermatogonial mitoses.

Circadian changes in the spermatogonia MI in intact rats was characterized by a circadian rhythm (Table 1). Smoothed curve of circadian dynamics of spermatogonia MI was a sinusoid with a 24-h period (Fig. 1, *a*), the active phase was observed from 3.00 to 12.00 during day 1 and from 6.00 to 12.00 on day 2. The acrophase of MI cycle was observed at 9.00 on both days. The amplitude of MI fluctuations was 45‰, the daytime MI value appreciably ($p<0.05$) surpassed the nocturnal MI (Table 1). Spectral analysis of the dynamics of proliferative activity of spermatogonia revealed a circadian rhythm of MI with a period of about 24 h and ultradian rhythm with a period of 7-8 h (Fig. 1, *b*).

After pinealectomy the number of type B spermatogonia increased ($p<0.01$; Table 1) and circadian rhythm of the spermatogonia MI disappeared. Smoothed curve of circadian changes in spermatogonia MI lost its sinusoidal shape (Fig. 2, *a*). On day 1 the active phase was observed during the light hours, while on day 2 it was observed during the dark hours and at the beginning of the light period. Shift of the acrophase was not regular. On day 1 it was observed at 12.00 (3-h lagging) and on day 2 at 6.00 (3-h anticipation). The amplitude of fluctuations in spermatogonia MI in pinealectomized animals increased to 60‰, but the mean MI values during the daytime and at night virtually did not differ. Spectral analysis showed only ultradian fluctuations of MI, their period was prolonged to 17 h (Fig. 2, *b*).

Injection of Epithalamin to pinealectomized animals restored the circadian rhythm of spermatogonia MI. Smoothed curve was characterized by sinusoidal fluctuations (Fig. 3, *a*). The active phase was observed during the light hours and lasted from 9.00 to 18.00 on day 1 and from 6.00 to 12.00 on day 2. Its acrophase was observed at 9.00 and 12.00 on days 1 and 2, respectively. On the other hand, the amplitude of spermatogonia MI fluctuations remained at a high level (71‰, *i.e.* similarly as after pinealectomy). Injection of Epithalamin caused a significant decrease of spermatogonia MI during the first hours (Fig. 3, *a*). The mean MI during the light hours was significantly

TABLE 1. Mitotic Index of Spermatogonia (‰, $M\pm m$)

Parameter	Group		
	control	pinealectomy	pinealectomy+ Epithalamin
Mesor	120.28±3.26	139.45±3.68	134.55±6.98
Day	127.98±2.48*	138.41±3.26	162.08±1.64*
Night	112.17±4.04	135.34±4.52	112.43±4.38

Note. *$p<0.01$ compared to night.

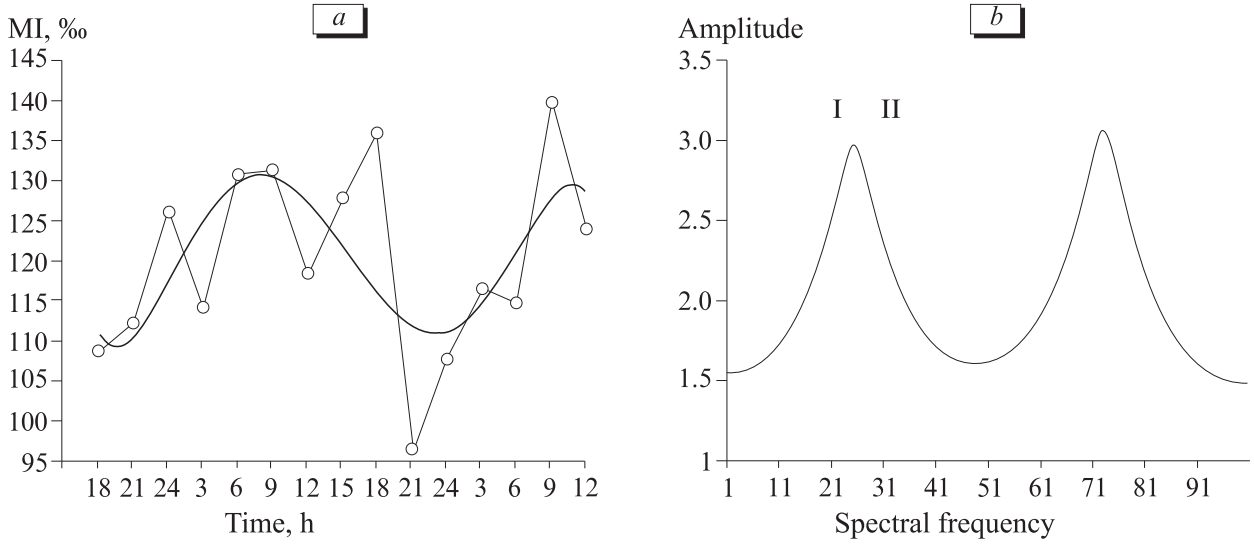

Fig. 1. Circadian dynamics of the mitotic index (MI) of spermatogonia in intact animals. Here and in Figs. 2, 3: *a*) smoothed curve; *b*) results of spectral analysis; I) circadian rhythm domain; II) ultradian rhythm domain.

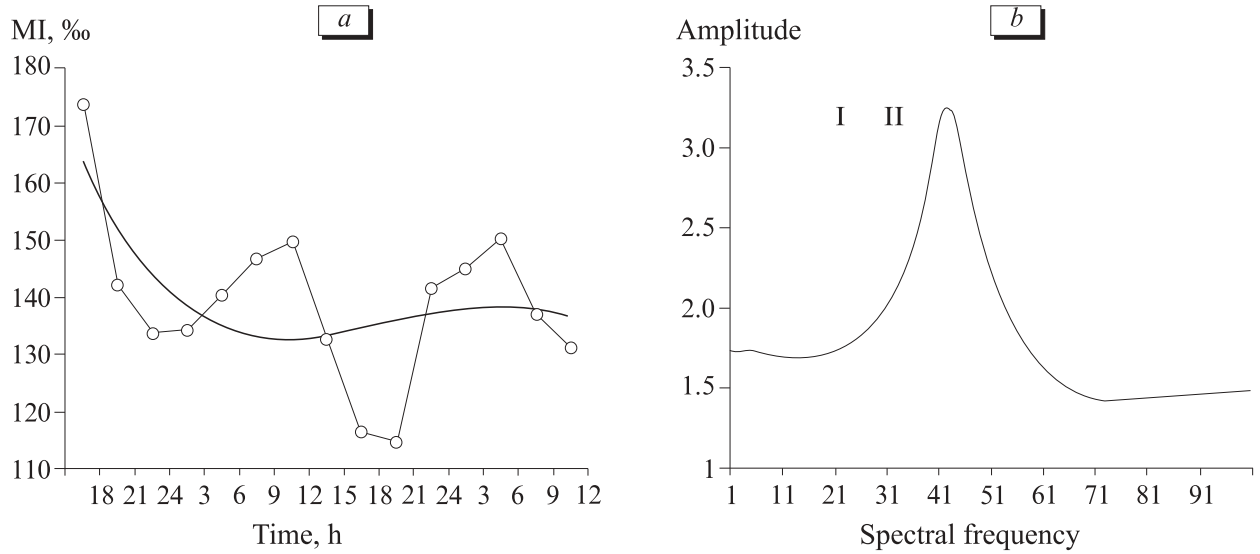

Fig. 2. Daily dynamics of spermatogonia MI in pinealectomized animals.

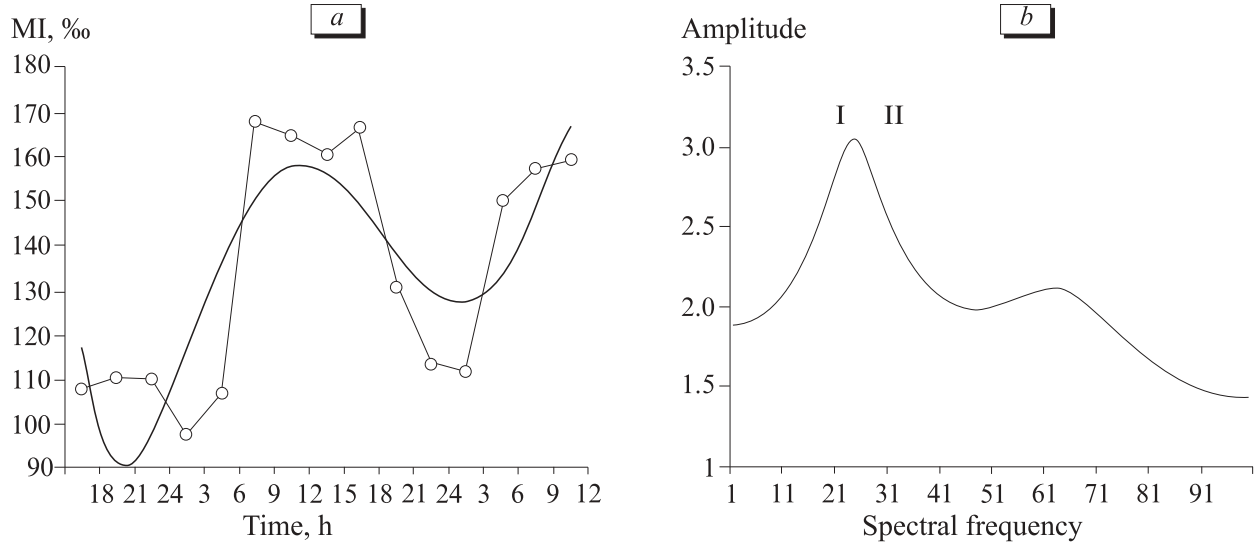

Fig. 3. Daily dynamics of spermatogonia MI in pinealectomized animals treated with Epithalamin.

higher than at night (Table 1). The mean 24-h MI value was significantly higher than in intact animals ($p<0.01$). Spectral analysis showed circadian rhythm of MI with a period of 24 h (Fig. 3, *b*). Hence, injection of Epithalamin to pinealectomized animals restored rhythmic organization of spermatogonia proliferation.

Spermatogenesis in albino rats takes 48 days, during which light and dark periods alternate. Adaptation of animals to photoperiodical changes manifest in the formation of circadian rhythms of body functions, *e.g.* spermatogenesis. Circadian rhythm of type B spermatogonia division is a manifestation of this adaptation. The process of spermatogenesis starts from division of spermatogonia. Presumably, circadian rhythm of the multiplication stage reflects rhythmic organization of spermatogenesis process in general, which is confirmed by circadian rhythm of follicle stimulating hormone and testosterone (regulation of spermatogenesis stages) production [6]. Disappearance of circadian rhythm of spermatogonia MI after pinealectomy attests to the involvement of the pineal gland in the regulation of their proliferation biorhythm by suppressing it during certain hours of the day. The inhibitory effect of melatonin on the proliferation of malignant trophoblastic cells *in vitro* and *in vivo* [7] and modulating effects of pineal peptides on the biorhythms of jejunal cryptic epithelial MI were shown [5]. Restoration of the circadian rhythm of spermatogonia MI after Epithalamin treatment indicates that pineal regulation of biorhythm of spermatogonia proliferation is realized not only through melatonin, but also through pineal oligopeptides. The pineal regulation of circadian changes in spermatogenesis cannot

be confined to the regulation of spermatogonia proliferation. The effect of the pineal gland on the daily changes in the blood levels of gonadotropic hormones and testosterone suggests the involvement of this gland in the regulation of daily dynamics of spermatogenesis in general.

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