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MORPHOLOGY AND PATHOMORPHOLOGY

Infradian Rhythm of the Content of Secretory Granules in Pinealocyte Cytoplasm in Mice and Rats

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The numerical density of secretory granules dense-core vesicles (DCV) in the cytoplasm of pinealocytes of the pineal gland was estimated by transmission electron microscopy in male white mice and Wistar rats. The 3-day biorhythm and lunaphase changes in the DCV content in the perikaryon and the processes of pinealocytes, which are manifested significantly in different seasons of the year, are established. The three-day biorhythm in adult male mice in comparison with younger male rats is not expressed uniformly in different phases of the moon. The in-phase manifestation of infradian biorhythms in different species of animals during the year with an unchanged daily photophase indicates the existence of common external synchronizers for mammals of these biorhythms that are not associated with the light/dark cycle.

Key Words: *pineal gland; biorhythms; ultrastructuremetry; mammals*

Pinealocytes of mammals and humans produce a wide range of hormones that have a regulatory effect on the diverse processes in the body [1]. Like hypothalamic neuroendocrine cells, they form a secreted vesicle with a dense core (DCV) in the Golgi complex that transport neurohormones from the perikaryon to the processes located near the capillaries. Indolamines serotonin and/or melatonin, as well as antigonadotropin and other biologically active polypeptides can accumulate in DCV. The ultrastructure of DCV has been studied in detail in various animal species [9,12,13]. It was demonstrated that pinealocytes do not store them in large quantities. Changes in the numerical density of DCV in the cytoplasm of pinealocytes correspond to shifts in the secretory activity of the pineal gland, which varies depending on circadian, circatriginntian and annual rhythms, age of animals, reproductive activity [4,5,7,10,12]. Information on the infradian rhythms of the functional activity of the pineal gland of other periodicity is not available in the susceptible literature. The three-day infradian biorhythm of concentration in the blood of thyroid hormones was found in male Wistar rats and chinchilla breeds during a period of intense growth [6]. The pineal gland affects the functional state of the organs of the pituitary—hypothalamic—thyroid system [2].

The goal of this work was to study infradian biorhythms of the DCV content in the cytoplasm of pinealocytes in mice and rats.

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MATERIALS AND METHODS

The work was performed on 3-month-old male white mice (CD1 sieve) weighing 25-30 g (n=135) and 4-month-old male Wistar rats weighing 180-200 g (n=150). The animals were kept in the photo mode at 12/12 h (light from 08.00 to 20.00 h) and were sacrificed daily for 27-30 days at 16.00 h for 5 indi-

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viduals: rats — in March-April, mice — in November-December. The animals were decapitated under ether anesthesia. The pineal glands were fixed in a mixture of 4% paraformaldehyde and 2.5% glutaraldehyde on 0.1M cacodylate buffer (pH 7.4), postfixed in 1% osmium tetroxide, dehydrated in ethanol and acetone, encapsulated in a mixture of epon—araldite resins. The slices were prepared on an ultratom of Leica EM UC7, contrasted with uranyl acetate and plumb citrate, and examined in a JEM-100 CX II (Jeol) transmission microscope. Pinealocytes that contain in the cytoplasm of DCV were photographed in 10 random fields of view of the pineal gland sections of each animal with an amplification of microscope in the ×14,000. The number and specific area of the profiles of the perikarya and processes of the pinealocytes were counted with 15×22.5 cm micrographs and a 368-point test-system. The average number of DCVs was calculated on the area of 100 μ^2 of the cytoplasm of pinealocytes [12].

Statistical processing of the results was conducted using Statistica 7.0 (StatSoft, Inc.). The median (Me), 25th and 75th quartiles (Q_1-Q_3) were calculated. The significance of the differences was assessed using the Mann—Whitney *U* test at $p<0.05$.

RESULTS

The numerical density of DCV in pinealocytes that reflects the functional state of the pineal gland, varied in different seasons of the year in different mammalian species in-phase, and exhibited 3-day and lunaphase shifts (Fig. 1, Table 1). On days 1-6 (phase of waning moon) and 7-13 (new moon), the content of DCV in pinealocyte cytoplasm exceeded that on days 14-20 (phase of growing moon) and 21-27 (full moon). In the phase of the growing moon in comparison with the phase of the waning moon, the changes in DCV content in pinealocyte cytoplasm were insignificant. On day 3 of the 3-day cycle, the content of DCV in mice often exceeded that on day 1, in rats — on day 2. On days 6 and 27 in mice and rats, the content of DCV in pinealocyte cytoplasm was higher than on

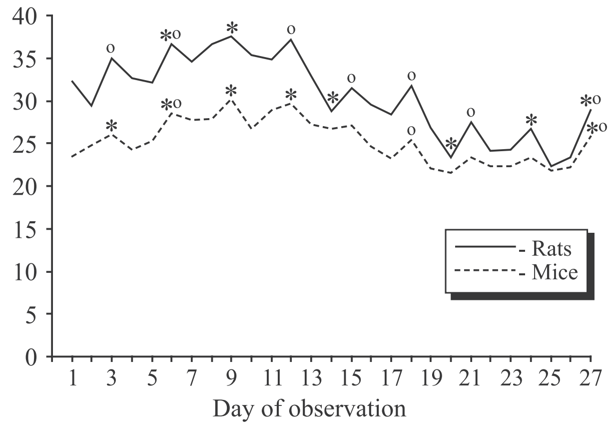

Fig. 1. Infradian rhythm of secretory vesicles with dense core (DCV) content in the pinealocytes of mice and rats. p<0.05 in comparison with *1 and °2 day of the 3-day period.

days 1 and 2 of the 3-day cycle. On days 13-15 and 19-24, no significant differences in the content of DCV pinealocyte cytoplasm were seen in mice. Given that all animals were kept at constant daily photophase, these shifts in the secretory activity of the pineal gland were not associated with the light/dark cycle. In accordance with the literature, their manifestation can be influenced by changes in the Earth's rotation speed around its axis, the solar magnetic activity that superimposed on the lunar cycle, the lunar gravity factor, and also the younger age of male rats in comparison with male mice [3,6,8,11]. Phase changes in the gravitational field of the moon, the rotation speed of the Earth around its axis, the magnetic activity of the sun, probably are perceived by pinealocytes. In-phase with external influences pinealocyte cytoplasm, vesicles with electron-dense grains move in mice, osmiophilic bodies — in rats. They are found at the ends of the processes of the pinealocytes and changed the size, shape, location of grains in their own matrix and microtubules of the cytoskeleton, as well as the length of synaptic bands [4,5,10]. Changes in the secretory activity of the pineal gland affect the functioning of other organs and systems of the body, which explains the appearance of the 3-day biorhythm of the con-

TABLE 1. Lunaphase Changes in the Content of Secretory Vesicles with Dense Core (DCV) (Me1 (Q_1-Q_3)

Phase	Number of DCV per 100 μ^2 pinealocyte cytoplasm	
	mice	rat
Waning moon (days 1-6)	24.8 (23.5-26.9)*°	32.8 (30.6-35.3)*°
New moon (days 7-13)	27.9 (26.8-29.7)°+	35.6 (33.9-37.4)°+
Growing moon (days 14-20)	24.3 (22.5-26.7)*°	28.8 (26.3-31.1)*°+
Full moon (days 21-27)	22.4 (21.4-23.9)*+	25.1 (23.4-27.5)*+

Note: *p*<0.05 in comparison with *the new moon, °full moon, +waning moon.

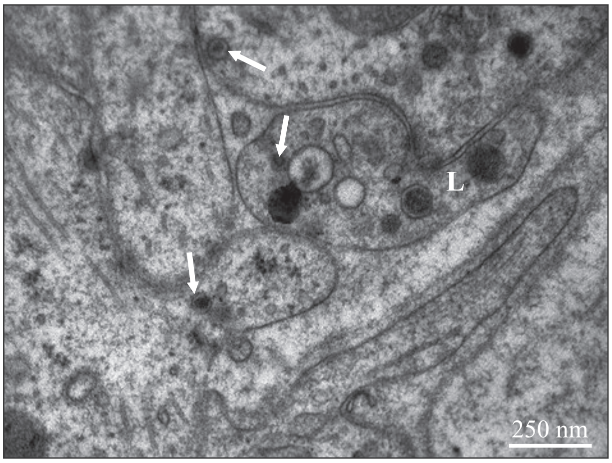

Fig. 2. Secretory vesicles with a dense core (arrows) in the perikaryon and processes of mouse pinealocytes on day 12, ×80,000 L: lysosome.

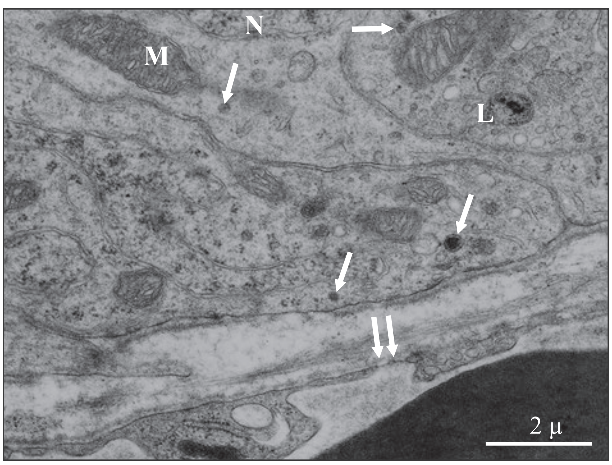

Fig. 3. Secretory vesicles with a dense core (arrows) in the perikaryon and processes of rat pinealocytes on day 14, ×14,000. N: pinealocyte nucleus, M: mitochondria, L: lysosome. Double arrow: fenestrated capillary endothelium.

centration of thyroid hormones in the blood in Wistar male mice and the lunaphase shifts in the functional state of the submaxillary glands in mice [4,6].

In addition to the specific differences of the pineal gland in the content of DCV in the cytoplasm of pinealocytes, the morphological manifestations of its secretory activity were found in mice and rats. A network of intercellular tubules develops in the gland of the mice that communicate with the pineal niche and the third ventricle of the brain, and the contents of DCV often released into the intercellular space. At the end of the new moon, in the perikaryon and claviform terminals of the processes of the pinealocytes, lysosomes are often found which, in accordance with [12], regulate “overproduction” of DCV in the Golgi complex. Thus, they “prepare” the body for a decrease in the level of functional activity in the phase of the growing moon and in the full moon (Fig. 2).

In rats, lysosomes are also involved in the regulation of secretory activity of pinealocytes. In addition, during the transition of the new moon to the growing moon phase, the specific volume of the Golgi complex pinealocyte cytoplasm decreases in rats and mice, the profiles of the Golgi sacs become shorter and wider, the lumen of the crista increases in the mitochondria. The processes of pinelaocytes with the claviform terminal are usually found in rats near the capillaries with the fenestrated endothelium rather than between the cells as in mice (Fig. 3).

The study showed that the pineal gland in male mice and rats displays 3-day biorhythm and lunaphase shifts of secretory activity. The three-day biorhythm of the content of secretory vesicles with a dense core in the pinealocyte cytoplasm in different phases of the moon, the changes in the phase of the diminishing and growing moon in adult male mice and younger male rats are not expressed in the same way. The in-phase manifestation of infradian biorhythms in different mammalian species observed in different seasons of the year with unchanged daily photophase indicates the existence of common external synchronizers of these biorhythms that are not associated with the light/dark cycle.

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