EXPERIMENTAL BIOLOGY

Effects of Various of Illumination Regimens on the Structure and Circadian Rhythms of Rat Epiphyseal Proteins in Postnatal Ontogeny

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It is demonstrated that the absence of the natural day/light cycle has no principal effects on the development of the pineal gland during the first two weeks of postnatal ontogeny. Starting from the 21st day, functional activity of the pineal decreases, particularly under conditions of constant illumination. The shift of acrophase testifies to relative structural stability of the investigated rhythm.

Key Words: *pinealocytes; protein metabolism; circadian rhythms*

Epiphysis is a unique organ whose structure and function are completely changed during the phylogeny of the vertebrates: from a photoreceptor organ to an neuroendocrine transducer [7,8].

Some researchers regard the pineal gland in mammals as a circadian biological "clock". At the present time the oscillator of circadian biological rhythms in mammals is often associated with the suprachiasmatic nucleus of the hypothalamus [9,10]. However, anatomical and functional relationships between epiphysis and hypothalamic-hypophyseal complex and retina as well as circadian rhythms in the contents of various biologically active substances revealed in the epiphysis and strong dependence of these rhythms on illumination regimen suggest that together with suprachiasmatic nucleus the pineal gland plays an important role in the development and adaptation of circadian biological rhythms [2,3,5,6]. In this context a thorough study of histogenesis and of the effect of illumination regimen on biological rhythms of the epiphysis [1,4] is interesting.

Our objective was to compare histogenesis and circadian rhythms of protein metabolism in pinealocytes (PC) of rats maintained under various conditions of illumination.

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MATERIALS AND METHODS

Epiphyses from male Wistar rats were collected on days 1, 7, 14, 21, 28, 56, and 84 of postnatal ontogeny. The animals were divided into three groups. They were maintained under conditions of natural illumination (group 1, control), constant illumination 1000 Lx (group 2), or constant darkness (group 3).

Experiments were carried out in December-February. To create experimental conditions from the moment of birth female rats were maintained on the constant illumination regimen with free access to food and water. For investigation of the structure of pineal gland rats were sacrificed at 12.00.

Epiphysis and its components were studied by histological (staining with hematoxylin and eosin and by Heidenhain's azane method) and neurohistological (Nissl method with I. V. Viktorov's modification) methods and by electron microscopy (ultrathin sections, EMB-100B and JEM-100CX microscopes).

For study of circadian rhythms of the total protein content and intensity of incorporation of labeled

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precursor in PC proteins, rats were sacrificed every 4 h during a 3-day period.

The intracellular protein content in PC was determined in an SMP-01 scanning microscope (Opton) by the intensity of staining of ultrathin sections (5 μ) with Fast Green (0.1%, pH 2.2). This parameter was calculated as the product of mean extinction and cell area.

The period, duration of acrophase, mesor, and other parameters of investigated rhythms were determined using the Kosinor-Spektr software.

RESULTS

Dark PC predominated in the pineal gland of newborn rats (8.3±1.4 %), light PC being located only in the central part of the pineal. Cell-to-cell contacts were represented by gap junctions and fusion zones. The intracellular protein content in PC was low (10.7±0.4 arb. units) and did not change within a 24-h period.

The number of light PC increased in the pineal gland of 7-day-old rats to 28.2±1.1%. In addition to gap junctions and fusion zones, the lock type cell-to-cell contacts were observed. There were circadian variations in the protein content, which was higher than in 1-day-old rats: 17.8±0.5 arb. units.

In 14-day-old rats light PC predominated (78.3± 0.9 %), while dark PA were located at the periphery of pineal gland. Cell-to-cell contacts were observed as gap junctions, fusion zones, and local thickenings of some areas of contacting plasma membranes with increased osmiophily. Circadian rhythms in protein content were recorded (Table 1).

The number of light PC and protein contents gradually increased from the 14th till the 84th day of ontogeny: up to 99.0±0.6% and 34.5±0.8 arb. units, respectively. Circadian rhythms in the protein content were observed in 14-, 21-, 28-, 56-, and 84-day-old rats (Table 1).

There were no differences in structure and protein metabolism of PC in experimental and control rats older than 14 days.

Compared with rats living under normal conditions, in rats living in constant illumination or constant darkness from the 14th till the 84th day of experiment functional activity of the pineal gland decreased, as evidenced by the number of light PA (32.8±1.2 and 66.8±1.0%, respectively) and protein content 27.5±0.4 and 30.9±0.1 arb. units, respectively).

In addition, the structure of circadian rhythms of protein content changed (Table 1).

Significant circadian rhythms in protein content were observed in rats living in conditions of constant illumination and darkness; however, the acrophase was not shifted considerably.

Rat pups can see on the 10th-12th day of life. This coincides with morphofunctional transformation of the pineal gland. Morphological maturation of PC and the formation of cell-to-cell contacts may account

TABLE 1. Circadian Rhythm in the Intraccelular Protein Content Rat Pinealocytes

Age, days	Day/night cycle			Constant illumination			Constant darkness		
	amplitude	acrophase, rad	period, h	amplitude	acrophase, rad	period, h	amplitude	acrophase, rad	period, h
14	1.8±0.3	4.2	22.9	2.5±0.5	3.9	25.7	3.0±0.1	4.3	25.7
15	2.1±0.2	3.9	25.7	3.3±0.4	4.0	24.8	2.6±0.1	4.6	26.7
16	2.2±0.2	4.1	23.9	2.8±04.	4.0	23.9	2.7±0.4	5.2	25.8
21	3.4±0.6	3.9	25.7	2.8±0.4	4.2	25.7	2.3±0.2	4.1	26.7
22	5.1±0.4	4.5	25.7	3.3±0.4	4.3	23.9	2.4±0.3	4.8	25.7
23	4.1±0.6	5.1	24.8	3.4±0.1	4.2	23.9	2.5±0.4	5.2	25.8
28	4.5±0.5	3.7	25.7	3.1±0.4	3.9	26.7	3.2±0.4	4.2	27.7
29	5.4±0.6	3.8	24.8	3.0±0.2	4.0	24.8	2.6±0.3	4.5	27.6
30	5.1±0.3	3.8	24.8	3.0±0.4	4.1	23.9	2.7±0.2	5.2	24.8
56	6.1±0.4	4.2	26.7	4.9±0.4	4.2	25.7	2.9±0.1	4.1	26.7
57	5.0±0.2	4.5	27.6	4.8±0.7	4.6	27.6	2.5±0.3	4.3	28.5
58	5.6±0.9	5.4	24.8	5.6±0.7	5.1	26.7	2.7±0.4	5.6	23.9
84	5.7±1.2	4.1	23.9	3.2±0.6	4.5	23.9	1.6±0.1	4.2	25.7
85	5.5±0.5	4.1	23.9	4.0±0.1	4.9	30.4	1.7±0.1	4.6	26.7
86	4.9±0.4	3.9	22.9	4.2±0.6	5.8	22.0	1.7±0.1	5.2	23.9

for synchronization of PC function, as evidenced by circadian rhythms in their total protein content.

Circadian rhythms in rats living in conditions of constant illumination indicate that the light-darkens cycle does no trigger the investigated rhythms immediately after birth and points to relative independence of these rhythms. The shift of acrophase in control and experimental animals points to the relativity of structural stability of the rhythm, which agrees with the concept that circadian rhythm is an adaptive process.

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