PHYSIOLOGY

Biosynthesis of Melatonin during Early Postnatal Ontogeny in Pineal Gland of Healthy Rats and Animals with Hereditary Retinal Degeneration

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The content of melatonin and its precursors in the pineal gland of healthy rats and rats with hereditary retinal degeneration is studied on days 20 and 40 of postnatal development. The measurements are performed between 02:30 and 03:00, when the content of melatonin is maximal. In December, the content of melatonin and N-acetylserotonin in 20-day-old rats with hereditary retinal degeneration is 1.9- and 3.2-fold higher than in healthy controls. The content of norepinephrine in the pineal gland of these rats is increased 1.8-fold. On postnatal day 40, no differences in the pineal content of indolamines are noted. An increase in the content of melatonin in 20-day-old rats with hereditary retinal degeneration in comparison with healthy controls is also observed in May. A decrease in the maximum nocturnal level of melatonin in May in comparison with December is shown both in rats with hereditary retinal degeneration (by 46%) and healthy controls (by 41%) only on postnatal day 40.

Key Words: *hereditary retinal degeneration; melatonin; pineal gland; seasonal changes*

The content of melatonin (MT) in the pineal gland and its concentration in the blood undergo cyclic changes. The rhythm of MT fluctuations in adult animals depends on the illumination conditions, while before postnatal day 20 it is determined by endogenous factors and does not depend on external stimuli [1]. The content of MT peaks during the nighttime and drops to minimum during daytime. There are also seasonal variations in MT rhythm, which are most pronounced at higher latitudes, where the circannual variations of the duration of night- and daytime are most pronounced. The period of high MT content in the pineal and blood has been shown to shrink in spring-summer [6]. The dynamics of the MT content in the pineal of golden hamster and in human blood also exhibits seasonal variations of the peak MT concentrations [6,7].

Bearing in mind the effect of illumination on the MT biosynthesis, it was interesting to investigate this process in rats with hereditary retinal degeneration (HRD) before and after blindness onset (on days 20 and 40 after birth, respectively). In rats, HRD is characterized by insufficient phagocytosis of the photoreceptor outer segments by retinal pigment epithelium. There is evidence that MT influences this process. The maintenance of a high blood concentration of MT results in accelerated separation of the apical fragments of photoreceptor outer segments [8]; experiments with tissue cultures have demonstrated that MT inhibits phagocytosis of polystyrene microspheres by pigment epithelial cells [5].

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MATERIALS AND METHODS

Experiments were carried out on Campbell (with HRD) and Wistar rats. In both rat strains melanin-aggregating activity of the blood appears at the same time of postnatal ontogeny [2]; therefore, Wistar rats can be used as a control. The animals were kept under natural illumination. The experiments were performed in December 1994 (light period from 11:00 to 16:00) and in May 1995 (light period from 06:00 to 22:00). It has been previously shown that the content of MT attains the maximum at 02:30-03:00 in both periods. The pineal glands were promptly removed under red illumination and homogenized in 0.54 N $HClO_4$. The homogenate was filtered through 0.45-μ filters, and the filtrate was analyzed by reverse-phase high-performance liquid chromatography with either fluorimetrical (for indolamines) or electrochemical (for norepinephrine) detection. Indolamines were separated on a Bondapak C_{18} column (15×2 mm, 10 μ). Tryptophan, serotonin, N-acetylserotonin, and 5-hydroxyindole-3-acetic acid (5-HIAA) were eluted from the column with a buffer containing 14.0 mM citric acid, 10.0 mM CH_3COOH, 1 mM Na_2-EDTA, and 0.04 mM sodium dodecyl sulfate, pH 3.5. Acetonitrile and methanol were added to the buffer at a ratio of 1:1:8 v/v. Measurements were carried out at excitation and emission wavelengths of 297 and 353 nm, respectively. The buffer for MT assay consisted of the same components (pH 3.2); the sensitivity of the method was increased by setting the photomultiplier at a high-sensitivity regime. Typical chromatograms are shown in Figs. 1 and 2.

The norepinephrine content was measured using a Lichrosorb C_{18} column (25×2 mm, 10 μ). The eluting buffer contained 0.03 M CH_3COONa, 0.135 M CH_3COOH, 0.1 mM Na_2-EDTA, and 0.04 mM sodium dodecyl sulfate. Methanol was added to the buffer at a ratio of 1:9. The oxidation potential of the detector was 0.75 V.

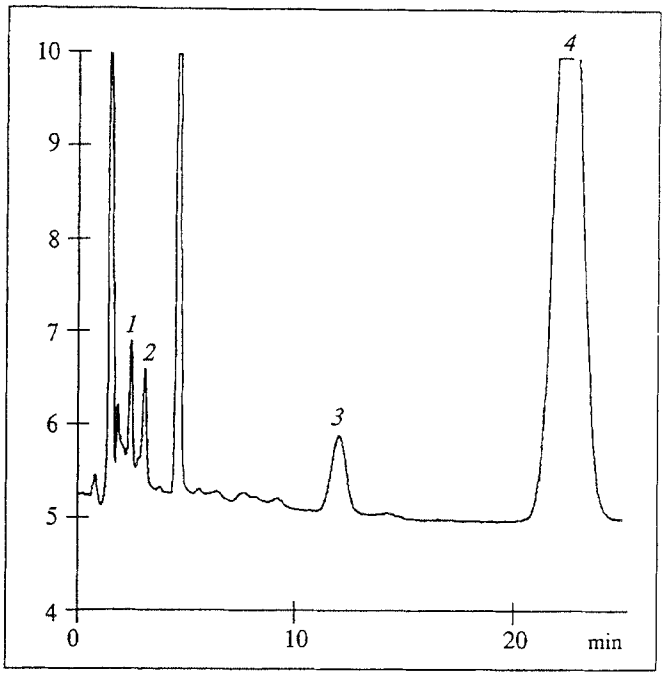

Fig. 1. Reverse-phase high-performance liquid chromatography (Bondapak C_{18}, 4×150 mm, 10 μ) of pineal homogenate, eluent pH 3.5. *1*) 5-hydroxyindole-3-acetic acid; *2*) N-acetylserotonin; *3*) tryptophan; *4*) serotonin.

RESULTS

The contents of tryptophan, MT, N-acetylserotonin, serotonin, 5-HIAA, and norepinephrine in the pineal gland of 20- and 40-day-old healthy and HRD-rats are shown in Table 1. In the pineal of 20-day-old HRD rats, the levels of MT and N-acetylserotonin increased 1.9- and 3.2-fold, respectively, compared with the control. This increase of both MT and its precursor N-acetylserotonin attests to the activation of serotonin-N-acetyltransferase, the key enzyme of MT biosynthesis. The elevated pineal content of norepinephrine in 20-day-old HRD-rats suggests that the enhanced synthesis of MT results from hyper-

TABLE 1. Content of Indolamines and Norepinephrine in the Pineal Gland (ng/Gland) of Wistar and Campbell rats on Postnatal Days 20 and 40 (*M*±*m*)

Parameter	Wistar		Campbell	
	20 days	40 days	20 days	40 days
Tryptophan	7.68±0.79 (7)	6.03±0.55 (6)	6.68±0.68 (7)	5.94±0.08 (6)
Serotonin	61.42±8.83 (7)	25.81±9.18 (6)	65.36±7.55 (7)	30.44±6.36 (6)
N-acetylserotonin	0.61±0.08 (7)	2.91±0.38 (6)	1.96±0.11 (7)**	3.09±0.68 (6)
MT	0.32±0.03 (10)	1.55±0.12 (6)	0.60±0.06 (10)*	1.34±0.14 (6)
5-HIAA	7.14±0.68 (7)	6.28±0.99 (6)	6.03±0.33 (7)	4.96±0.18 (6)
Norepinephrine	3.55±0.38 (6)	—	6.54±0.68 (6)*	—

Note. The number of experiments is shown in parentheses. *$p<0.01$, **$p<0.001$ in comparison with the control. Dash: not measured.

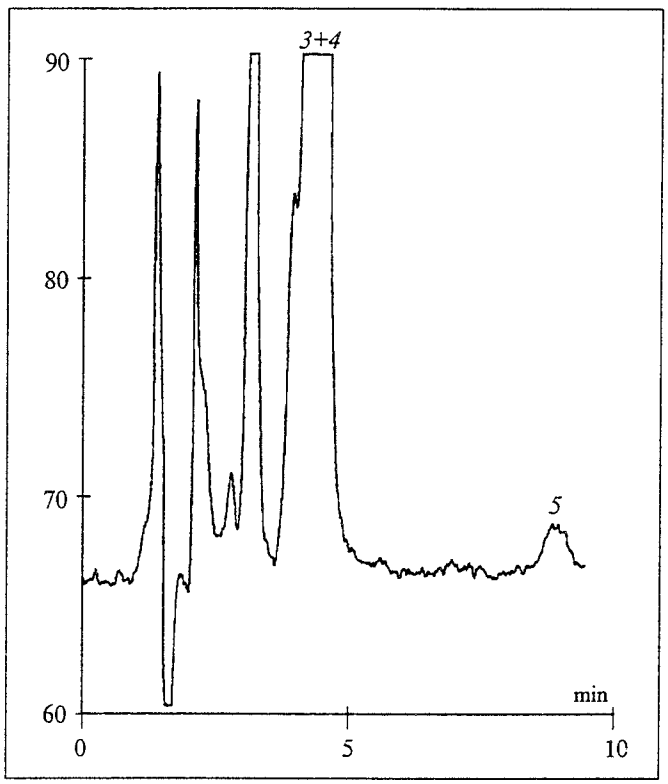

Fig. 2. Reverse-phase high-performance liquid chromatography (Bondapak C_{18}, 4×150 mm, 10 μ) of pineal homogenate, eluent pH 3.2. *3+4*) serotonin and tryptophan; *5*) melatonin.

stimulation of pinealocytes by norepinephrinergic neurons of the superior cervical ganglion. In adult animals, the activity of these neurons depends on illumination conditions and determines the intensity of MT biosynthesis in the pineal gland [4]. However, it should be noted that the observed elevation of the pineal MT content cannot be a consequence of developing blindness, since the MT cyclicity in the pineal gland on postnatal day 20 does not depend on illumination conditions [1].

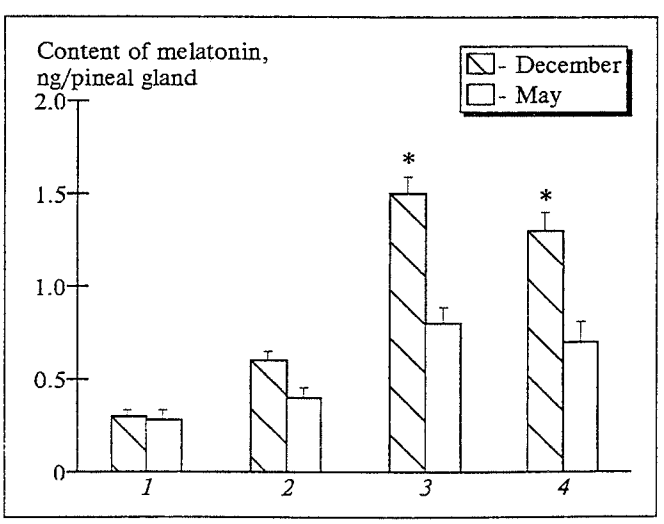

Fig. 3. Seasonal variation of pineal melatonin content in 20- (*1*, *2*) and 40-day-old (*3*, *4*) Campbell rats (*2*, *4*) with hereditary retinal degeneration and Wistar rats (*1*, *3*). *Seasonal changes are statistically significant at $p<0.01$.

No differences in the content of the studied substances were found on postnatal day 40. It has been previously shown that blindness leads to a drop in the serotonin-N-acetyltransferase activity [3]. Presumably, blindness of Campbell rats on postnatal day 40 is responsible for the absence of the difference in the pineal MT content.

Seasonal variations of the MT content in the pineal gland are shown in Fig. 3. Both in healthy animals and HRD rats on postnatal day 40 the nocturnal content of MT in the pineal dropped in May compared with that in December. These changes are related to considerable shortening of nighttime in spring and summer at the St. Petersburg latitude. Interestingly, seasonal variation also occurred in blind 40-day-old HRD rats. This suggests that the MT rhythm is established from postnatal day 20 to 35, when vision was partially preserved. The absence of seasonal variations on postnatal day 20 confirms that the MT rhythm at this period does not depend on illumination conditions.

The higher content of MT in the pineal of HRD rats probably results from a more rapid functional maturation of the pineal in comparison with the control. Our preliminary experiments showed that as early as on postnatal day 25 the MT content in the pineal gland of Wistar rats attains the level observed in 40-day-old animals (1.17±0.12 ng/gland in May). Thus, even a slightly accelerated development of the MT-synthesizing function of the pineal may lead to marked changes in the MT content.

Blood concentration of MT mainly depends on the rate of its synthesis in the pineal gland. Therefore, the content of MT in 20-day-old HRD rats is probably elevated. When injected into the blood, MT is able to accumulate in the eye [9]. It is likely that in HRD MT promotes degenerative processes in the retina by inhibiting phagocytosis of apical fragments of the photoreceptor outer segments by pigmented epithelium.

Concerning the regulation of MT biosynthesis in the early postnatal ontogeny, the effect of endogenous factors on this process requires further investigation.

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The Role of Dopaminergic System in the Immunostimulatory Effects of Substance P and Its Analog

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Systemic administration of synthetic substance P or its analog EC-1 to CBA mice results in considerable stimulation of immune reactions. No stimulation is observed after disconnection of the hypothalamus from the pituitary. It is concluded that the immunostimulating effect of these peptides is mediated by the dopaminergic system, since it is abolished by the D_2 receptor blocker haloperidol.

Key Words: *neuropeptides; immunostimulation; dopaminergic system; pituitary*

It is known that substance P stimulates both T and B cells [6,13]. On the other hand, this substance may have a role in the etiology and pathogenesis of various mental disorders and in stress reactions [3,11]. Several analogs of substance P exhibit biological activity in the central nervous system [10]. However, the neurochemical mechanisms by which tachykinins (for example, substance P) modulate immunogenesis remain obscure. Functional interactions between P-peptidergic terminals and dopamine (DA) cell bodies have been demonstrated in various brain structures [5,15]. The receptors for substance P have been identified in the substantia nigra and neostriatum [15]. In the present study we attempted to evaluate the contribution of the interactions between tachykinins and dopaminergic system to the immune response.

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MATERIALS AND METHODS

CBA mice (n=161, body weight 20-22 g) were used. Substance P (Vektor) or its analog EC-1 (a generous gift of Dr. O. S. Papsuevich from the Institute of Organic Chemistry, Riga) were injected in doses of 1, 10, and 100 μg/kg 30 min before immunization. The mice were treated with 2 mg/kg haloperidol (Gedeon Richter) 2 h before the antigen administration. All substances were injected intraperitoneally in 0.2 ml of physiological saline. In experiments with more than one test substance, the interval between injections was 10 min. The mice were immunized with sheep erythrocytes (5×10^8 cells, intravenously). The immune response was evaluated by counting plaque-forming cells [7] and rosette-forming cells (RFC) [2] on days 4 and 5 postimmunization, respectively.

The pituitary stalk was destroyed transauricularly under Nembutal anesthesia (50 mg/kg) 10 days

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