EXPERIMENTAL BIOLOGY

Circadian Rhythms of *In Vitro* Incorporation of ^{3}H-Melatonin into Organs of the Hypothalamic-Pituitary-Thyroid System in Rats

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Circadian rhythms of the intensity of ^{3}H-melatonin absorption by organs of the hypothalamic-pituitary-thyroid system were studied in young mature male Wistar rats in summer. The hypothalamus, pituitary, and thyroid gland incorporated ^{3}H-melatonin during the day and night hours, but the intensity of its incorporation in the pituitary and thyroid was high during daytime and low at night, while absorption of the hormone by the hypothalamus tended to be higher at night.

Key Words: *hypothalamic-pituitary-thyroid system; melatonin; circadian rhythms*

Close cooperation exists between the pineal gland and the hypothalamic-pituitary-thyroid system (HPTS). A peculiar feature of the pineal gland hormone melatonin (MT) is that normally its inhibitory effect is observed at all levels of HPTS functioning: injection of MT inhibits the formation of thyrotropin-releasing hormone in the hypothalamus, of thyrotropin in the pituitary, and thyroid hormones in the thyroid gland, which suggests the central (mediated) effect of MT on thyroid activity. However, *in vivo* and *in vitro* experiments demonstrate principal possibility of direct (also inhibitory) effect of MT on intrathyroid hormone production [2,8, 15]. Melatonin modulates virtually all stages of thyroid hormone biosynthesis and metabolism, including iodine capture and incorporation in thyrocytes, its organification, synthesis and secretion of thyroxine and its metabolic transformations; the inhibitory effect of MT on triiodothyronine secretion is less pronounced.

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The regulatory effect of MT on HPTS is realized due to the presence of specific MT receptors on the outer membrane of hormone-producing cells of this system [7]. Being a "nocturnal hormone" [6], MT exhibits its regulatory effect on the formation of circadian rhythms, including rhythms of endocrine glands, predominantly during the night hours [4,5], while the overwhelming majority of studies are carried out during the daytime, which precludes objective evaluation of the biological effects of MT.

We studied circadian peculiarities of MT incorporation into HPTS organs in rats.

MATERIALS AND METHODS

The study was carried out in summer (July) on young mature male Wistar rats (n=20). The animals were kept under standard vivarium conditions at natural illumination.

The animals were sacrificed at noon (period of minimum activity of the pineal gland) at natural sunlight (group 1, n=10) and at midnight (period of

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TABLE 1. Circadian Dynamics of ^{3}H-MT Binding by HPTS Organs in Adult Rats *In Vitro* (*M*±*m*)

Group	Time of the day	^{3}H-MT incorporatin, cpm/organ		
		hypothalamus	pituitary	thyroid
1	Day	512.80±26.08	580.70±11.03	2228.55±78.95
2	Night	569.25±10.52	172.41±7.96**	726.28±28.12*

Note. *p<0.05, **p<0.001 compared to group 1.

maximum activity of the organ, group 2, n=10) at red light, which virtually did not modulate the MT-producing function of pinealocytes. All manipulations on animals were carried out in accordance with the "Common Ethics of Experiments on Animals" [1]. The hypothalamus, pituitary, and thyroid gland were removed, weighed, placed into medium 199 with 0.01 μCi ^{3}H-MT (NEN Products), and incubated for 1 h at 37°C. After removal and 5-fold washout, the tissue specimens were placed into flasks with scintillation fluid. The intensity of ^{3}H-MT incorporation was evaluated on a BETA-2 scintillation counter and expressed in cpm/organ.

The data were processed statistically using Student's t test.

RESULTS

The hypothalamus, pituitary, and thyroid glands incorporated ^{3}H-MT during the daytime and at night, but with different intensity (Table 1). Binding of ^{3}H-MT by the pituitary and thyroid gland at night was lower than during daytime (by 3.6 and 1.6 times, respectively). On the other hand, a trend to a more intense accumulation of ^{3}H-MT by the hypothalamus during the night hours was observed. This means that the target endocrine glands, such as the pituitary and thyroid gland, having specific MT receptors on the membranes of hormone-producing cells, are characterized by clear-cut circadian rhythm of MT binding from circulation, while the hypothalamus, which also selectively binds MT, "behaves" differently at night. Hence, although HPTS is characterized as an integral system, studies of the mechanisms regulating hormonal activity of the thyroid gland showed different circadian rhythms of MT binding by the endocrine glands.

These data indicate that specific MT receptors on thyrocyte membranes are mainly occupied at night, *i.e.* at the peak of endogenous MT biosynthesis, release, and blood content, while during the daytime, when the concentration of endogenous MT drops, the number of vacant binding sites sharply increases. These data are in line with the results indicating that ^{125}I-MT binding decreased in the presence of unlabeled hormone [3].

In the pituitary, a similar but even more pronounced 24-h dynamics of MT binding to receptors was observed.

Since the intensity of ^{3}H-MT binding in the hypothalamus at night tended to increase, there are good grounds to expect that the number of vacant MT receptors in general will increase in this organ at night. The hypothalamus is a very intricate neuroendocrine structure, including numerous so-called nuclei regulating the endocrine (and some other) functions. Autoradiography of the 24-h distribution of MT binding sites is to be carried out individually for each group of nuclei.

Hence, the hypothalamus, pituitary, and thyroid gland selectively bind circulating ^{3}H-MT during day and night hours, the intensity of the hormone incorporation in the pituitary and thyroid being high during the daytime and low at night. Accumulation of ^{3}H-MT in the hypothalamus slightly varies within 24 h with a trend to an increase at night.

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