Intensity of *In Vitro* Incorporation of ^{3}H-Melatonin in the Thyroid Gland of Rabbits with Pineal Gland Hypofunction

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The structure and hormonal activity of the thyroid gland and its capacity to bind ^{3}H-melatonin were studied in young adult Chinchilla rabbits with pineal gland hypofunction induced by 2-month exposure to constant illumination. After 2 months of constant exposure to light, the experimental animals exhibited signs of the so-called "latent hypothyroidism" and more intense binding of ^{3}H-melatonin by the thyroid gland. This fact indicated intactness of its receptor system underlying the possibility of restoring activity of the thyroid gland under conditions of melatonin replacement therapy in hypothyroidism induced by chronic melatonin insufficiency.

Key Words: *hypofunction of the pineal gland; hypothyroidism; melatonin; thyroid gland*

Our previous studies on adult male rabbits showed that long-term exposure to constant illumination led to the development of acute melatonin insufficiency under conditions of progressive destruction of pinealocyte structure. This exposure should be regarded as a method of simulation of involution processes in the pineal gland or as an experimental model of hypofunction of the pineal gland [2].

Normally, the pineal gland regulates activity of the thyroid gland (TG) through melatonin due to the presence of melatonin receptors binding the hormone circulating in the blood [6,9]. Our experiments showed that hypofunction of the pineal gland induced by long-term continuous light exposure preventing the formation of the nocturnal melatonin peak was associated with the development of phasic changes in hormonal activity of TG, characterized by initial (during the first month of light exposure) intensification of biosynthesis and secretion of total thyroxin (T_4) and triiodothyronine (T_3) with their subsequent (after 3-5 months) reduction and development of hypothyroidism of the neuroendocrine nature. The term of 2 months after the beginning of hypofunction of the pineal gland induction was particularly interesting because of the contradictory data: histological signs of TG morphology and function exhaustion were already present in TG after preliminary overstrain, while hormonal analysis showed normalization of blood levels of T_3 and T_4. These data suggested that the initial stage of hypothyroidism, or the so-called "latent hypothyroidism", was detected during that period. Simultaneous measurement of free thyroid hormones would have been more informative in this aspect, but we failed to find these data in published reports.

Melatonin deficiency in hypofunction of the pineal gland is corrected by substitution therapy [4,5]. This hormone not only prolongs the life span [4,5], but also reduces the toxicity and stimulates the efficiency of other drugs in combined therapy [7]. It remains unclear whether the capacity of TG (weakened in hypofunction of the pineal gland) to bind circulating melatonin is unchanged.

Hence, we studied ^{3}H-melatonin binding by TG in rabbits with hypofunction of the pineal gland.

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MATERIALS AND METHODS

The study was carried out on 32 adult male Chinchilla rabbits. Controls were kept at natural light, experimental animals in which hypofunction of the pineal gland was induced were exposed to continuous illumination. During the daytime they were exposed to natural sun light, at night to electric light (100 Wt lamp). The level of illumination in the cells was 30-40 Lx. The experiment was carried out during 2 months.

The animals were sacrificed in accordance with the regulations of the European Convention on the Protection of Vertebrates Used for Experimental and Other Research Purposes (Strasbourg, 1985). Hormonal activity of TG was evaluated by measuring the serum concentrations of total and free T_3 and T_4 by enzyme immunoassay using Alcor Bio and DRG standard kits on a Stat Fax 2100 EIA analyzer. In order to evaluate the morphology and function of TG, the organs in some rabbits were fixed in 10% formalin immediately after removal, processed in ascending alcohols, and embedded in paraffin, after which 5-μ sections were sliced and stained by hematoxylin and eosin. The micropreparations were examined under a Jenaval microscope (Carl Zeiss). In other rabbits, TG was weighed, weighed material (20-25 mg) was plunged in medium 199 with 0.01 μCi ^{3}H-MT (NEN Products) and incubated for 1 h at 37°C. The specimens from control and experimental rabbits were then washed 5 times and put into flasks with scintillation fluid. The intensity of ^{3}H-MT incorporation was evaluated on a BETA-2 scintillation counter and expressed in cpm/mg tissue.

Analysis of normal distribution of signs was carried out by parametric methods. The groups were compared using Student's *t* test.

RESULTS

The concentrations of total and free T_3 increased significantly during 1 month of constant light exposure. Total T_4 exhibited a trend to increase, free T_4 virtually did not change; these facts attest to more intense hormonal activity of TG during this period (Table 1). These data agree with the concept on the antithyroid effect of melatonin [6]. On the other hand, after 2 months the total T_3 and T_4 levels returned to normal and did not statistically differ from the initial values, while the concentration of free T_3 dropped in comparison with the previous period ($p<0.01$) and the initial level ($p<0.01$). This was paralleled by a trend to reduction of free T_4 level.

As we know, just a negligible portion of T_3 and T_4 (0.3% T_3 and 0.02% T_4) circulate in the free form in the blood, but these forms are directly involved in realization of many regulatory functions in humans and animals [8] and hence, measurements of these hormone forms serve as more adequate and direct markers of TG hormonal activity. These very hormone values indicated a probable manifestation of hypothyroidism 2 months after the beginning of hypofunction of the pineal gland induction.

Histological studies confirmed this hypothesis. TG of young adult intact rabbits had a histological structure characteristic of this animal species, described in literature (Fig. 1, *a*).

TG of rabbits exposed to constant illumination over 2 months exhibited clear-cut signs of functional inhibition during this period, which could be regarded as exhaustion after long (1 month) overstrain (Fig. 1, *b*). These signs consisted a drastic (2-3-fold) dilatation of follicles overfilled by homogeneous compact colloid, low (flat) follicular epithelium, no Sanderson pads. All these phenomena indicated that inhibition of hormonal activity of the pineal gland in experimental rabbits led to the development of all histological signs of hypothyroidism after 2 months. However, microscopy showed some sites of intact TG parenchyma in the visual field during this period.

Comparison of the hormonal and histological findings after 2 months of constant light exposure in

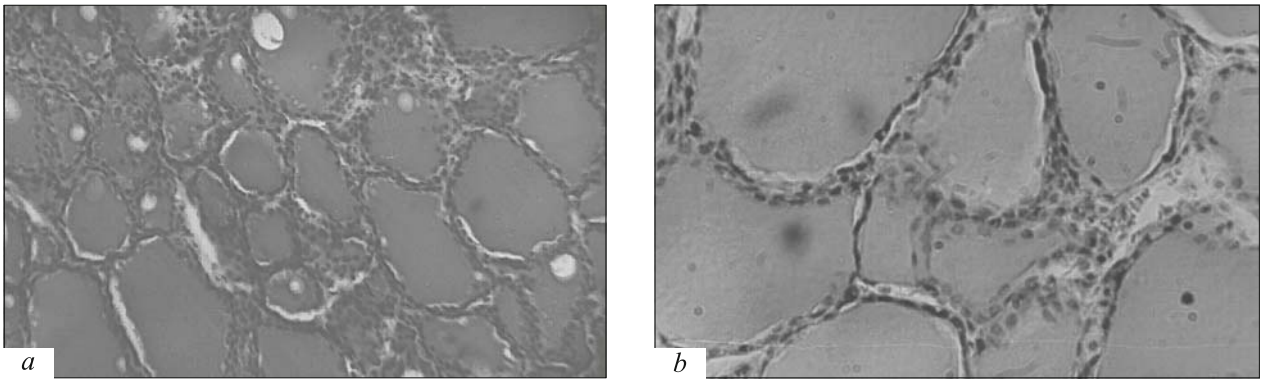

Fig. 1. Histostructure of TG in a young adult rabbit: intact (*a*) and after 2-month continuous illumination (*b*). Hematoxylin and eosin staining, ×320.

TABLE 1. Blood Concentrations of Total and Free Thyroid Hormones in Rabbits with Pineal Gland Hypofunction Induced by Exposure to Constant Illumination ($\bar{X}\pm S_X$)

Experimental series	Experiment conditions	Blood hormone concentration			
		total T_3, nmol/liter	free T_3, pmol/liter	total T_4, nmol/liter	free T_4, pmol/liter
I	Initial status (natural day/night)	1.21±0.09 (n=15)	7.11±0.30 (n=7)	47.85±1.95 (n=15)	21.46±1.45 (n=7)
II	Continuous illumination (1 month)	2.06±0.09*** (n=15)	9.66±0.25*** (n=7)	53.79±2.09* (n=15)	18.89±0.87 (n=7)
III	Continuous illumination (2 months)	1.24±0.08^{+++} (n=15)	5.19±0.21**$^{+++}$ (n=7)	44.74±2.27^{++} (n=15)	17.70±1.24* (n=7)

Note. *0.05<p<0.1, **p<0.01, ***p<0.001 compared to series I; ^{++}p<0.01, ^{+++}p<0.001 compared to series II.

experimental rabbits attested to the development of the so-called "latent" hypothyroidism, which could be diagnosed during life time only by measuring the free forms of thyroid hormones.

The data characterizing the intensity of ^{3}H-MT incorporation in TG of experimental rabbits are summed up in Table 2.

The intensity of ^{3}H-MT uptake by TG increased (p<0.02) in experimental rabbits after 2 months of constant light exposure, reaching 118.1% of the control (100%). These data indicated that TG under conditions of chronic melatonin insufficiency not only retained the capacity to bind melatonin circulating in the blood after 2 months of constant illumination, but did it even more intensely. The results indicated that the melatonin binding receptor system of TG parenchyma remained intact in experimental animals during this period. It could be expected that later (after 3-5 months) TG of rabbits with hypofunction of the pineal gland would still bind this hormone, because, according to our findings, the course of melatonin replacement therapy could restore the structure and hormonal activity of the organ in these animals [3].

The results indicate that the intensity of ^{3}H-MT incorporation in TG exhausted in hypofunction of the pineal gland increased, presumably due to the increase in the number of melatonin receptors on thyrocyte membrane. The physiological efficiency of this process was presumably explained by the need in restoration of the double neuroendocrine regulation of TG: by the pituitary gland (through thyrotropin) and pineal gland (through melatonin).

TABLE 2. Effects of 2-Month Continuous Illumination on the Intensity of ^{3}H-MT Incorporation in TG of Adult Rabbits ($\bar{X}\pm S_X$)

Experiment conditions	Intensity of ^{3}H-MT uptake, cpm/mg wet tissue
Natural day/night (n=5)	243.00±14.70
Continuous illumination (n=5)	287.00±3.37**

Note. **p<0.02 compared to natural day/night illumination.

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