Specific Binding of ^{3}H-Melatonin to Cell Plasma Membranes in Rat Thyroid Gland

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^{3}H-melatonin binding sites were found in the cell membrane fraction from the thyroid gland of Wistar rats and their kinetic characteristics were determined. The number of receptors and dissociation constant depend on the duration of daytime, which probably determines rhythmic fluctuation of the sensitivity of thyrocytes to melatonin.

Key Words: *epiphysis; melatonin; membranes; thyroid gland; cAMP*

Since epiphyseal hormone melatonin (MT) plays a great role in neurohumoral regulation, the mechanisms of its effect on endocrine glands and the existence of specific receptors on target organs remain an important problem. The effect of MT was long thought to be mediated through hypothalamic structures. This assumption was confirmed by identification of MT specific binding sites in rat and hamster brain, in particular in the suprachiasmatic nucleus [2,8]. However, radioligand assay with the use of ^{125}I and ^{3}H-MT demonstrated the presence of MT receptors not only in different brain areas and in the pituitary, but also in peripheral endocrine glands [4,5]. There are no data on the presence of MT in thyrocytes; however, the existence of these receptors is quite possible, since the thyroid gland is one of the main target organs for MT [6,10]. The assumption that MT receptors are localized in the thyrocyte membrane arises from previous experimental and clinical studies [1,6] demonstrating a relationship between the effect of MT and accumulation of cAMP, i.e., activity of membrane bound enzyme adenylate cyclase. It is particularly interesting to determine the number of MT receptors on the thyrocyte membrane and their dissociation constant (K_d) in different illumination regimes, since the peripheral effect of MT strongly depends on the sensitivity of target cells characterized by circadian and circannual rhythms [5].

The objective of the present study was to identify MT binding sites on thyrocyte membranes and to determine their kinetic characteristics.

MATERIALS AND METHODS

Experiments were carried out on thyroid glands from 98 male Wistar rats maintained either under natural illumination with long daytime (summer, series I) or in 24-h darkness (series II). Specific binding of MT to the thyrocyte membranes was studied on crude membrane fraction from the thyroid gland. To this end, the thyroid glands were removed, stripped off surrounding tissues, washed in physiological solution prepared on phosphate buffer (pH 7.5) at 4°C. All manipulations were performed on the cold. A 10-fold (w/w) excess of isolation buffer containing 10 mM imidazole-HCl (pH 7.5) and 1 mM EDTA was added, and the tissue was homogenized in a Polytron homogenizer. The homogenate was filtered through 4-fold gauze and centrifuged at 6000*g* for 10 min, and the pellet was washed with the isolation buffer. The final pellet was suspended in the isolation buffer in the following proportion: thyroid glands from 10 animals per 1 ml buffer; 1-ml aliquots were stored in liquid nitrogen. The binding of ^{3}H-MT (46.9 Ci/mmol, NEN) with thyrocyte membranes was assessed as described previously [2] with some modifications.

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To this end, membranes (200-300 μg protein) were incubated for 5 h at 4°C in 0.5 ml incubation medium containing 10 mM imidazole-HCl (pH 7.5) and 1 mM EDTA, 10^{-10}-10^{-7} M ^{3}H-MT. Nonspecific binding was measured in the presence of 10^{-3} M unlabeled MT (Sigma). The reaction was terminated by rapid dilution with an excess of incubation buffer (10 mM imidazole-HCl, pH 7.5, and 1 mM EDTA), and the mixture was filtered through GF/C filters (Whatman) in an MR-24 filtration system (Brandel). The filters were washed 4 times with 5 ml buffer and transferred to counting vials with 5 ml scintillation mixture (Atomlight, NEN) and counted on a Rack-beta counter (LKB). Specific binding was calculated by subtraction the nonspecific binding from the total count.

The data were processed on IBM using Binding software.

RESULTS

Binding assay with various concentration of labeled MT demonstrated that the formation of ^{3}H-MT-receptor complexes is a concentration-dependent saturable process.

The binding of ^{3}H-MT to thyrocyte membranes from rats maintained under natural illumination (summer) attained the plateau at a hormone concentration of 12 nM and peaked at 5×10^{-8} M. Scatchard analysis revealed the existence of two types of MT binding sites in the thyrocyte membranes (Fig. 1). The number of high-affinity binding sites (K_d=2.2×10^{-10} M) was 2.0 pmol/mg membrane protein, while the number of low-affinity binding sites (K_d=5.5×10^{-9} M) was 17.0 pmol/mg protein, suggesting heterogeneity of MT receptors in the thyrocyte membrane. Binding of MT to thyrocyte membranes suggests that MT exerts direct membrane-coupled effect on the thyroid gland; however, the presence of MT receptors in other cell structures cannot be excluded.

In rats maintained in the darkness for 24 h, saturation of MT binding sites was attained at a lower concentration of the hormone (6 nM) (Fig. 2). The number of high-affinity binding sites (K_d=5.4×10^{-10} M) increased approximately 2-fold, being 3.8 pmol/mg protein. The population of low-affinity binding sites with high binding capacity diverge to 2 subtypes: 5.7 pmol/mg protein MT binding sites with K_d=1.2×10^{-9} M (subtype I) and 10 pmol/mg protein binding sites with K_d=21.4×10^{-9} M (subtype II).

These experiments demonstrated a rise of the density of MT binding sites in rats maintained in the darkness and a possibility of partial intraconversion of low-affinity receptors into a "high-affinity" state. N. Zisapel [11] hypothesized that the high-affinity

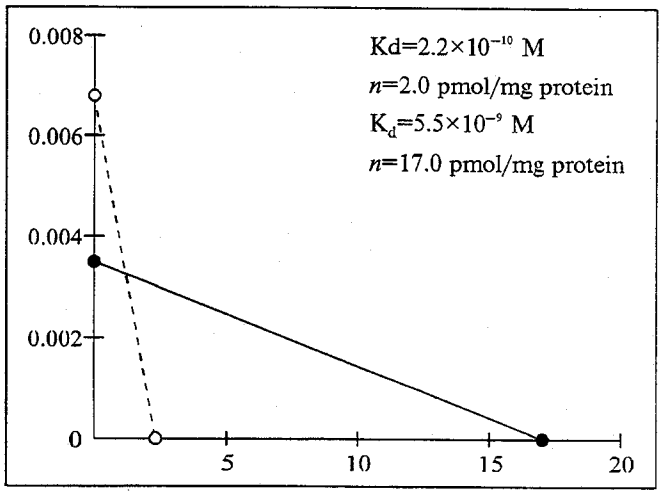

Fig. 1. Scatchard plot of ^{3}H-melatonin specific binding to thyrocyte membranes from rats maintained under natural illumination (long daytime). Here and on Fig. 2: abscissa: bound melatonin, pmol/mg protein; ordinate: bound/free ^{3}H-melatonin.

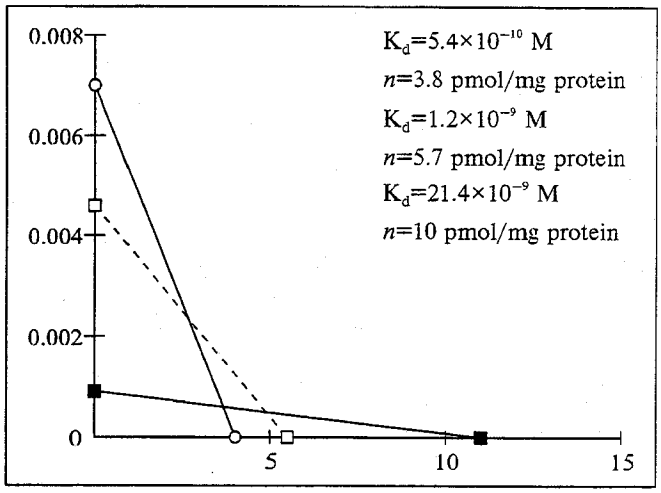

Fig. 2. Scatchard plot of ^{3}H-melatonin specific binding to thyrocyte membranes from rats maintained in darkness.

state is characteristic of desensitized MT receptors, therefore the observed changes in the ^{3}H-MT binding kinetics in animals maintained in the darkness to a some extent explain the well-known inefficiency of morning injection of the pineal hormone.

It can be assumed that the increased number of MT receptors (also due to intraconversion) and their enhanced binding capacity are adaptive phenomena. For instance, in the suprachiasmatic nucleus only a single type of binding sites was detected in the nighttime, while in 12:00 h a two-component binding was observed with the presence of low- and high-affinity MT binding sites; in the brain from rats sacrificed on 7:00 h no MT binding sites were found [8,9]. The authors believed that this reflects the circadian rhythms of receptor affinity.

We have no information regarding the time of MT receptor formation in the thyroid gland. How-

ever, we believe that these receptors already function at birth, since our previous experiments [6] showed that injection of MT into newborn rats modulates the response of the thyroid gland to thyrotropic hormone in 1-month-old rat pups.

Thus, our findings suggest that the effect of MT on thyrocytes is realized through a transmembrane pathway. This effect is most probably associated with the light/dark cycle and represents an adaptive phenomenon.

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Ontogenetic Dynamics of Arterial Pressure and ECG in NISAG Rats

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Ontogenetic changes in cardiac function, relative heart mass, and arterial pressure occurring in rats with genetically determined arterial hypertension (NISAG) are compared with those occurring in normotensive rats (Wistar). In NISAG rats hypertension is accompanied by shift of electrical axis of the heart to the left, increased heart rate, cardiac conductance disturbances, and relative coronary insufficiency. It is suggested that in NISAG rats changes in ECG are secondary to arterial hypertension.

Key Words: *genetically-determined arterial hypertension; heart; electrocardiography; ontogenesis*

NISAG rats with stress-sensitive arterial hypertension were bred by selection at the Institute of Cytology and Genetics (Siberian Division of the Russian Academy of Sciences) [11]. Previously, it was shown that adult NISAG rats with high arterial pressure (AP) develop typical changes in the cardiovascular system. These changes are hypertrophy of left heart [3], ECG deviations typical of relative coronary insufficiency, proneness to arrhythmias, and formation of necrotic foci in the myocardium in response to epinephrine [4].

In this study we examined ontogenetic changes in AP and cardiac function in NISAG rats.

MATERIALS AND METHODS

Male NISAG (hypertensive) and Wistar (normotensive) rats aging 1, 2, 3, and 6 months were used. Arterial pressure was measured sphygmographically on the tail [6]. Electrocardiogram was recorded with a Mingograph-34 cardiograph using needle electrodes. The electrodes were inserted subcutaneously

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