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Research report

The regional and cellular expression profile of the melatonin receptor MT1 in the central dopaminergic system

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Abstract

The physiological effects of pineal melatonin are primarily mediated by melatonin receptors located in the brain and periphery. Even though there are a number of studies demonstrating the regulatory role of melatonin in the development of dopaminergic behaviors, such as psychostimulant-induced diurnal locomotor sensitization or drug seeking, little is known about the contribution of melatonin receptors (i.e., MT1) to this role. Therefore, as a first step in understanding the functional role of melatonin receptors in dopaminergic behaviors, we focused on determining the expression pattern of MT1 receptors in the dopaminergic system of the human and rodent brain. Regional (e.g., nucleus accumbens shell) and cellular (e.g., tyrosine hydroxylase immunopositive cells) expression of MT1 mRNA was characterized by applying the immuno-laser capture microdissection (immuno-LCM) technique coupled with nested RT-PCR. Moreover, employing quantitative Western immunoblotting and RT-PCR, we found that the mouse MT1 receptor expression presents diurnal variations (i.e., low mRNA and high protein levels at night, ZT21). The dopaminergic system-based presence of MT1 receptor proteins was not limited to rodents; we found these receptors in postmortem human brain as well. Further research is needed to understand the regional/cellular functional role of melatonin receptors in the regulation of dopaminergic behaviors, using models such as melatonin receptor knockout mice.
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Topic: Regional localization of receptors and transmitters

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1. Introduction

In pineal gland neurohormones, N-acetylserotonin (NAS) and melatonin are synthesized from serotonin via the rate-limiting enzyme arylalkylamine N-acetyltransferase (AANAT). Upon synthesis, NAS and melatonin are secreted into the blood circulation and cerebrospinal fluid in a circadian fashion, that is, with high levels at night [12]. NAS and melatonin produce their effects primarily through melatonin receptors, MT1 (Mel1a) and MT2 (Mel1b) [see reviews in Refs. [20 and 49]]. MT1 and MT2 receptors have been cloned and identified as members of the seven-transmembrane G protein-coupled receptor family [20,34,36]. Studies using specific agonists and antagonists demonstrate that melatonin receptors are involved in the antinociceptive, antidepressant, anxiolytic, antineophobic, and locomotor activity modulatory effects of melatonin [18,24,27,30,32,53].

The putative role of melatonin in the dopaminergic system has been suggested based on studies demonstrating the effect of melatonin on dopamine content [3], turnover [2,4,39], and release [9,16,56] as well as on dopamine receptor (i.e., D1 and D2) activation [14,15]. Moreover,

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based on using melatonin treatment, diurnal rhythms, pinealectomy, and melatonin-proficient and -deficient inbred mice strains, it has been suggested that melatonin regulates the development of dopaminergic system-mediated addictive behaviors such as cocaine-induced locomotor sensitization and reward [1,19,41,47,48]. It is possible that melatonin receptor-mediated cellular mechanisms (i.e., cAMP-mediated) maybe involved in this regulatory effect of melatonin. In fact, it has been demonstrated that the cAMP pathway (i.e., cAMP/PKA/CREB) and the target genes regulated via this pathway, such as Cdk5, modulate the development of cocaine-induced behaviors including sensitization and reward [8,22,25,26].

Although the expression of melatonin receptors has been shown in the rodent brain (MT1 in hippocampus, cortex, hypothalamus, and cerebellum [5,21,31,34,42] and MT2 in cerebellum and hippocampus [5,21,34]), there is no information regarding the regional/cellular expression and further, the expression pattern of these receptors in the dopaminergic system. Here, for the first time, we report combined data on the expression dynamics of the melatonin receptor MT1 in this system, using the immuno-laser capture microdissection technique coupled with nested RT-PCR (for regional and/or cellular expression patterns) along with quantitative RT-PCR and Western immunoblotting techniques (for daily variations in mRNA and protein levels).

2. Materials and methods

2.1. Subjects and sample preparation

2.1.1. Rodent studies

C3H/HeJ mice (male, 8 weeks old and weighing 25–30 g) and Sprague–Dawley rats (male, 8 weeks old and weighing 250–300 g) were purchased from Jackson Laboratories (Bar Harbor, ME) and Harlan (Indianapolis, IN), respectively. Animals were housed in groups of three and had free access to laboratory chow and water. They were kept in a temperature-controlled room under conditions of 14 h light:10 h dark cycle (lights on at 5 am, ZT00). The experimental protocol was approved by the Institutional Animal Care Committee. To perform the immuno-laser capture microdissection, after a 10-day acclimatization period, anesthetized mice and rats were perfused transcardially with either 0.9% ice-cold sterile saline or paraformaldehyde (4%), depending on the type of antibody that was used in initial immunohistochemistry step until the outflow from the right atrium was clear. The brains were removed and frozen immediately on dry ice and stored at −80 °C until they were processed to protect the RNA. On the other hand, to measure the daily expression levels of MT1 receptors (i.e., mRNA expression and protein levels), dopaminergic regions from these mice were excised from 1-mm-thick coronal brain slices as described elsewhere [45], both during the day (ZT05) and at night (ZT21), and were stored at −80 °C. Sample size was 4 to 6 per time point for both mRNA and protein studies. Sample collections at night were performed under dim red light (0.1 lx at the bench level). We choose the sample collection time based on the circulating melatonin levels for these inbred mice, light cycles, and our previous experiments on cocaine-induced behavioral changes [12,47,48].

2.1.2. Human studies

Postmortem human brains (ranging 12 to 16 h postmortem interval time) were obtained from normal control subjects who died of natural causes. Brain samples were processed as previously described in detail [10] and stored at −80 °C until used to detect MT1 receptor proteins. It has been reported that samples processed with a similar range of postmortem interval and kept in the same storage conditions had been successfully used for both RT-PCR and Western immunoblotting studies [10]. To limit the variables such as postmortem interval time for each individual, we analyzed brain region samples from the same individual (i.e., 16 h postmortem interval). The experiment was repeated in another set of brain regions from a different individual with 12 h postmortem interval time.

2.2. Immunohistochemistry and laser capture microdissection (immuno-LCM) with nested RT-PCR

2.2.1. Immuno-LCM

Mouse or rat brains were cut coronally into either 10- (for immunohistochemistry/cellular dissection) or 20- (for regional dissection) μM thick sections in the cryostat and mounted directly on Leica slides for membrane-based laser microdissection. For regional dissections, areas of dorsomedial caudate, dorsolateral caudate, olfactory tubercle, nucleus accumbens shell and core, substantia nigra, and ventral tegmental area were identified based on cellular organization observed under the microscope and the coordinates from the mouse brain atlas [28]. For this purpose, sections were fixed in ethanol briefly, stained with toluidine blue (Sigma, St. Louis, MO), and immediately examined under a micro-dissection microscope. For each region, approximately six to 10 sections were dissected and collected.

To collect the immunolabeled cells positive for D2 receptors and for tyrosine hydroxylase, sections from mouse and rat brains were fixed in ice-cold ethanol for 5 min at −20 °C to protect the integrity of the epitopes for efficient antibody–protein interactions. For tyrosine hydroxylase immunolabeling, we have used the same exact conditions (e.g., the commercial monoclonal antibody to detect tyrosine hydroxylase and the method of histochemistry for this particular immuno-LCM technique) reported by Backes and Hemby [6]. Since the antibody we used in our studies to detect tyrosine hydroxylase (Sigma, St. Louis, MO, cat #T-1299) works in rats but not in mice [6],

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