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Evidence for feedback control of pineal melatonin secretion

Tracy A. Bedrosian*, Kamillya L. Herring, James C. Walton, Laura K. Fonken, Zachary M. Weil, Randy J. Nelson

Department of Neuroscience, The Ohio State University Wexner Medical Center, Columbus, OH 43210, USA

HIGHLIGHTS

- Luzindole injection increases serum melatonin concentrations.
- 4-P-PDOT, an MT2 specific antagonist, does not affect serum melatonin.
- Possible feedback control of pineal melatonin secretion, occurring through MT1.

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ABSTRACT

Melatonin is the principle hormonal product of the pineal gland. It is secreted with a robust daily rhythm, peaking near the middle of the night. During the daytime, concentrations remain very low, as exposure to light robustly suppresses its secretion. The regulation of melatonin by light is well-characterized, but an interesting feature of the daily melatonin rhythm is that its peak occurs near the middle of the night and then levels begin to drop hours before morning light exposure. The mechanism underlying the light-independent drop in melatonin during late night remains unspecified. Feedback control is one mechanism of hormone regulation, but no studies thus far have explored the possibility of such regulation in the pineal of white-footed mice (*Peromyscus leucopus*). The pineal gland and SCN express melatonin receptors, and melatonin regulates its own receptor density in the brain. We investigated the possibility of feedback control of melatonin by administering melatonin receptor antagonists to female white-footed mice and then measuring plasma melatonin concentrations. In the first experiment, we observed that luzindole, a dual MT1/MT2 receptor antagonist administered 1 h after lights off, caused an increase in plasma melatonin both 1 and 2 h later. In a second experiment, we did not observe a change in melatonin concentrations following injection of an antagonist specific for the MT2 subtype. These results suggest the possibility of feedback control of melatonin release, occurring preferentially through the MT1 receptor subtype.

1. Introduction

Melatonin is the principle hormonal product of the pineal gland [20]. It is secreted with a robust daily rhythm, peaking near the middle of the night. During the daytime, concentrations remain very low, as exposure to light robustly suppresses its secretion in an intensity-dependent manner [14]. In humans and other mammals, detection of light drives activity in retinal ganglion cells that project to the suprachiasmatic nucleus (SCN) in the hypothalamus, causing the release of inhibitory GABA that suppresses the circuit controlling melatonin synthesis and release. In darkness, the SCN synaptically evokes noradrenalin release from the superior cervical ganglion (SCG). Noradrenalin in turn acts on β-adrenergic receptors in the pineal to provoke melatonin synthesis and secretion.

The regulation of melatonin by light is well-characterized, but an interesting feature of the daily melatonin rhythm in humans and other mammals is that its peak occurs near the middle of the night and then levels begin to drop hours before morning light exposure [16]. Evidence shows that a daily rhythm exists in expression of serotonin N-acetyltransferase (NAT), an enzyme involved in converting serotonin into melatonin via N-acetylserotonin (NAS), that contributes to the daily rhythm in melatonin production [18]. Control of NAT varies by species, in some cases gene expression of NAT is turned on at night through a cAMP-dependent process that is regulated by light information [3]. In other species, NAT is regulated at the protein level [12]. These examples reveal how light modulates melatonin production upstream at the synthetic enzyme level, but recently it was demonstrated that NAT continues to build up at

* Corresponding author at: Department of Neuroscience, The Ohio State University Wexner Medical Center, 636 Biomedical Research Tower, 460 W. 12th Avenue, Columbus, OH 43210, USA. Tel.: +1 614 688 4674; fax: +1 614 292 3464.
E-mail address: Bedrosian.2@osu.edu (T.A. Bedrosian).

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very high levels even throughout late night, yet does not produce a proportional increase in melatonin concentrations [13]. The mechanism underlying the light-independent drop in melatonin during late night is not fully understood.

Feedback control of hormone secretion is a primary mechanism of neuroendocrine regulation. Thus far, no evidence exists to demonstrate feedback control of melatonin secretion, but melatonin receptors are present in the pineal gland of several species, including humans, rodents, and birds [2,17,22]. Melatonin can regulate the expression levels of its own receptors in the SCN of rats. After either several days of exposure to constant light or pinealectomy, melatonin receptor density is robustly increased, but an injection of melatonin rapidly reverses this effect within 4 h [10]. It is possible that melatonin levels build during early night, providing feedback to the pineal gland to eventually shut off production near the middle of the night, resulting in the observed drop in melatonin concentration occurring during late night.

We investigated this hypothesis by administering melatonin receptor antagonists to female white-footed mice and then measuring plasma melatonin concentrations during the dark phase. In the first experiment, we observed the effects of luzindole, a dual MT1/MT2 receptor antagonist, on plasma melatonin concentrations. In a second experiment, we assayed melatonin concentrations following injection of an antagonist specific for the MT2 subtype.

2. Methods

2.1. Animals

Adult female white-footed mice (*Peromyscus leucopus*) were obtained from our breeding colony at The Ohio State University. Mice were group-housed in polypropylene cages (32 cm × 18 cm × 14 cm) at constant ambient temperature (22 ± 2 °C) and relative humidity (50 ± 5%) and provided *ad libitum* access to food (Harlan Teklad 8640, Indianapolis, IN, USA) and filtered tap water. Mice were kept on a 16 h:8 h light–dark cycle, with lights out at 15:00 h.

2.2. Injections

Mice were injected under dim red light 1 h after lights off with a melatonin receptor antagonist or vehicle. In the first experiment, mice were injected i.p. with 0.1 ml of 30 mg/kg luzindole (Sigma) dissolved in 95% ethanol or 0.1 ml of 95% ethanol. In the second experiment, mice were injected i.p. with 90 μg of 4P-P-DOT (Tocris Bioscience, Bristol, UK) dissolved in 0.1 ml of 30% ethanol or 0.1 ml of 30% ethanol alone.

2.3. Blood sampling

In each experiment, mice were randomly assigned to blood sampling either 1 h or 2 h post-injection. Blood samples were collected under dim red light from the retro-orbital sinus through a heparinized capillary tube, kept in the dark, and immediately centrifuged for 15 min at 1800 × *g*, then plasma was aliquoted and stored at −80 °C until melatonin assay.

2.4. Melatonin assay

Frozen plasma samples were thawed on ice and assayed for melatonin concentrations using a commercially available double antibody RIA kit according to the manufacturer's instructions (#01-RK-MEL2, Alpco Immunoassays, Salem, NH, USA). The intra-assay coefficient of variation was <10%.

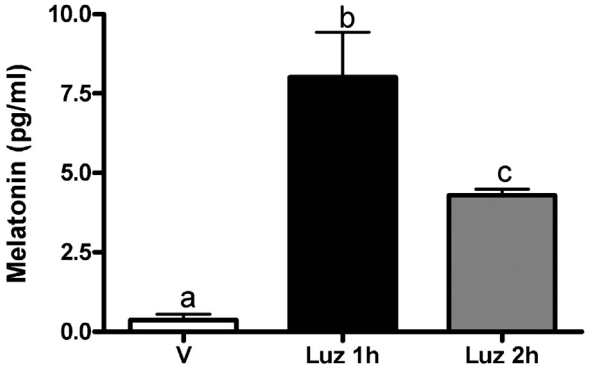

Fig. 1. Plasma melatonin concentrations 1 and 2 h after luzindole injection. Different letters denote that all bars are statistically different from one another ($p < 0.05$). $n = 6–8$ per group.

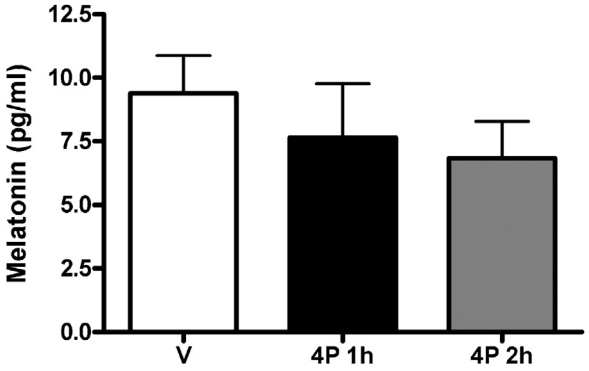

Fig. 2. Plasma melatonin concentrations 1 and 2 h after 4-P-PDOT injection. $n = 8–10$ per group.

2.5. Statistics

Vehicle treated groups 1 h and 2 h post-injection were collapsed in both experiments because they did not differ statistically. One-way ANOVA was used to compare treatment groups and main effects were followed with Fisher's post hoc tests where applicable. Data were analyzed using Statview 5.0.1 (SAS Institute, Cary, NC, USA) for Windows. Mean differences were considered statistically significant when p was ≤ 0.05.

3. Results

3.1. Luzindole

Treatment with the dual MT1/MT2 receptor antagonist luzindole significantly increased plasma melatonin concentrations compared to vehicle treatment ($F_{2,16} = 15.950$, $p < 0.001$), both 1 h (post hoc, $p < 0.0001$) and 2 h ($p < 0.05$) post-injection (Fig. 1). Concentrations were greater 1 h post-injection compared to 2-h (post hoc, $p < 0.01$).

3.2. 4-P-PDOT

Treatment with the selective MT2 receptor antagonist 4-P-PDOT did not significantly influence melatonin concentrations at either 1 h or 2 h post-injection ($p > 0.05$; Fig. 2).

4. Discussion

Melatonin concentrations tend to peak during the middle of the night, and then decrease during the late night before any morning light exposure. Light is clearly a robust regulator of melatonin

secretion, but the mechanism for this downswing during late night remains unexplained. White-footed mice differ from traditional laboratory strains of mice in that they produce robust rhythms of pineal melatonin and express both MT1 and MT2 receptor subtypes [15,21], making them a useful model for these experiments. White-footed mice also represent a genetically heterogeneous group, potentially making them a more naturalistic model. We injected luzindole, a dual MT1/MT2 receptor antagonist 1 h after lights off, and observed increased plasma melatonin concentrations 1 and 2 h later compared to vehicle-treated mice. Though luzindole acts at both receptors, it has approximately 11- to 25-fold greater affinity for the MT2 receptor [1,6]. In contrast, after injection of 4-P-PDOT, we did not observe any change in melatonin concentrations. 4-P-PDOT is a much more selective antagonist at the MT2 subtype, with >300-fold preference. These results suggest that MT1 may mediate the increased melatonin concentrations following injection.

One surprising finding was the difference between assays in the melatonin concentrations of vehicle-treated mice. Melatonin concentrations were much higher in the second experiment, and one explanation may be the different vehicle treatments for each experiment. Because of the solubility of the drugs in ethanol, mice were injected with a greater concentration of ethanol in the first experiment compared to the second. Ethanol robustly and rapidly suppresses melatonin levels in a dose-dependent manner [7,19], which may explain the reduced plasma melatonin concentrations in the first experiment.

Converging mechanisms may contribute to the decline in melatonin concentrations during late night. For example, noradrenergic input to the rat pineal gland shows a robust daily rhythm that peaks at night and correlates with the rhythm of pineal melatonin release, suggesting that rhythmic sympathetic input drives the nightly melatonin rhythm [5]. Without noradrenergic stimulation, intracellular cAMP levels drop [11], leading to a decrease in AANAT activity [9]. Beta adrenergic receptors also become desensitized during the late night [8]. And finally, reduced substrate availability could potentially result in reduced melatonin synthesis. By administering melatonin antagonists during the upswing of melatonin levels in early night, we likely avoided any interaction with additional mechanisms that could preclude our ability to specifically detect melatonin receptor-mediated effects. In support of our results, exogenous melatonin reduces the amplitude of endogenous melatonin measured by *in vivo* microdialysis in a rat model [4], though this effect was not observed in sheep [23].

Overall, our results point toward the possibility of feedback control of pineal melatonin secretion through the MT1 receptor in white-footed mice, but more investigation is necessary to definitively support this hypothesis.

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