Pinealectomy Does Not Affect Diurnal PER2 Expression in the Rat Limbic Forebrain

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Number of text pages: 14
Number of Figures: 1

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Acknowledgements

Supported by grants from the Canadian Institutes of Health Research, the Natural Science and Engineering Council of Canada, and the Concordia Research Chairs Program.

Abstract

A role for the pineal hormone, melatonin, in the regulation of the rhythmic expression of circadian clock genes is suggested by the finding that surgical removal of the pineal gland abolishes the rhythm of expression of clock genes such as Per1 in several neural and endocrine tissues in rodents, including the caudate-putamen and nucleus accumbens, the hypophyseal pars tuberalis, and adrenal cortex. Pinealectomy has no effect on clock gene rhythms in the suprachiasmatic nucleus (SCN), the master circadian clock, as well as in the eyes and heart, indicating the effect of melatonin on clock gene rhythms is tissue specific. To further study the role of melatonin in the regulation of the rhythm of clock genes, we assessed in rats the effect of pinealectomy on the rhythm of expression of the clock protein, PER2, in a number of key limbic forebrain structures, the oval nucleus of the bed nucleus of the stria terminalis (BNST-OV), the central nucleus of the amygdala (CEA) and the hippocampus (HIPP). Despite previous evidence showing that these regions are sensitive to melatonin, pinealectomy had no effect on the daily rhythm of expression of PER2 within these structures, further supporting the view that the role of endogenous melatonin in the regulation of clock gene expression is tissue specific.

Keywords: Pineal gland, Oval nucleus of the bed nucleus of the stria terminalis, Central nucleus of the amygdala, Hippocampus, Caudate-putamen, suprachiasmatic nucleus, PER2, Diurnal rhythm, rat

The synthesis and release of the pineal hormone melatonin follows a daily rhythm that is regulated by the environmental light cycle and the circadian clock located in the suprachiasmatic nucleus (SCN) [14, 19, 20] and there is considerable evidence to suggest that melatonin participates in the regulation of circadian rhythms. For example, the SCN contains high levels of MT1 and MT2 melatonin receptors [10, 21], and treatment with melatonin has been shown to modulate the rhythm of electrical activity of SCN neurons in vitro [22-24]. Moreover, systemic administration of melatonin has been shown to entrain circadian behavioral rhythms as well as to modulate the effect of the light cycle on circadian rhythms in vivo [2, 18].

Melatonin has also been implicated in the regulation of the expression of clock genes responsible for circadian rhythm generation at the cellular level. Specifically, it has been shown that the pineal gland is essential for the rhythm of expression of the clock gene Per1 in a number of neural structures and endocrine tissues, including the caudate-putamen (CP) and nucleus accumbens, the hypophyseal pars tuberalis, and adrenal cortex [7, 11, 28, 29, 31]. In contrast, it was found that removal of the pineal gland has no effect on the rhythmic expression of the clock genes, Per1 and Per2 in the SCN, eyes and heart [11, 17], suggesting that the effect of melatonin on clock gene expression is tissue specific.

We recently found that the clock protein, PER2, is expressed rhythmically in a several key limbic forebrain structures in the rat. These include the oval

nucleus of the bed nucleus of the stria terminalis (BNST-OV), the central nucleus of the amygdala (CEA) and the hippocampus (HIPP) [1, 8]. To study the role of melatonin in the regulation of clock gene rhythms in these areas, we assessed the effect of pinealectomy on PER2 rhythms in the BNST-OV, CEA and HIPP in rats. In addition, we assessed the effect of pinealectomy on PER2 expression in the CP, a region shown previously to exhibit pineal-dependent rhythms in Per1 gene expression in mice [29].

The experimental procedures followed the guidelines of the Canadian Council on Animal Care and were approved by the Animal Care Committee, Concordia University. A total of 12 pinealectomized (PINX) and 12 sham-operated (SHAM) male Sprague Dawley rats (Hsd:SD, 4 weeks old, 50-75g) were purchased from Harlan (Indianapolis, Indiana). They were housed two per cage in a temperature- and humidity-controlled room under a normal 12h:12h light-dark (LD) cycle (300 lux at cage level) and had free access to food and water. One month later they were transferred to individual cages housed in ventilated, sound and light tight isolation chambers and kept under a 12h:12h LD cycle with free access to food and water. One month later, the rats were injected with an overdose of sodium pentobarbital (~100 mg/kg) at one of four zeitgeber times (ZT1, ZT7, ZT13, ZT19; ZT0 denotes time of light on) and were perfused intracardially with 300 ml of cold saline (0.9% NaCl) followed by 300 ml of cold, 4% paraformaldehyde in a 0.1 M phosphate buffer (pH 7.3). Following perfusion, brains were postfixed in 4% paraformaldehyde and stored at 4° C overnight.

Serial coronal brain sections (50 µm) containing regions of interest (SCN, BNST, amygdala, hippocampus, caudate-putamen) were collected from each animal using a vibratome. Immunocytochemistry for PER2 was performed as previously described [1] using an affinity purified rabbit polyclonal antibody raised against PER2 (ADI, San Antonio, TX). PER2-stained brain sections were examined under a light microscope and images were captured using a Sony XC-77 video camera, a Scion LG-3 frame grabber, and Image SXM software (v1.6, S D Barrett, http://www.ImageSXM.org.uk). Cells immunopositive for PER2 were counted manually using the captured images. For analysis, the mean number of PER2 immunoreactive cells per region was calculated for each animal from the counts of 6 unilateral images showing the highest number of labeled nuclei. Differences between groups were revealed with analysis of variance (ANOVA). Alpha level was set at 0.05 for all analyses.

Examples of PER2 expression in the SCN, BNST-OV, CEA, HIPP and CP of SHAM rats killed at ZT1 or ZT13 and graphs showing the effect of pinealectomy on PER2 rhythms in each of these regions are shown in Fig. 1. PER2 expression in the SCN, BNST-OV and CEA peaked at ZT13 as previously shown. Furthermore, consistent with previous reports, pinealectomy had no effect on PER2 expression in the SCN ($F[1,16]=.251$, $p=.623$). Similarly there were no differences in PER2 expression in the BNST-OV ($F[1,16]=.24$, $p=.71$) and CEA ($F[1,16]=.18$, $p=.67$) between SHAM and PINX rats. In the HIPP PER2 expression peaked at ZT1 and, as was the case with the SCN, BNST-OV and

CEA, pinealectomy had no effect on PER2 expression in HIPP ($F[1,16]=1.415$, $p=.251$). Finally, PER2 expression in the CP peaked at ZT1. However, unlike the blunting effect of pinealectomy reported previously for striatal Per1 mRNA and PER1 expression in mice [29], pinealectomy had no effect on PER2 expression in this region in the rat ($F[1,16]=.196$, $p=.664$).

A role for melatonin in the regulation of the rhythm of expression of Per1 in the pituitary gland, caudate-putamen and nucleus accumbens is suggested by the evidence that pinealectomy and genetic deletion of melatonin receptors abolish the circadian rhythm of expression of Per1 mRNA and protein in these tissues in mice and hamsters [7, 11, 29]. Pinealectomy was shown not to affect rhythmic Per1 and Per2 expression in the mouse SCN and in the SCN, eye and heart in rats [11, 17], indicating that the effect of melatonin on the rhythm of expression of clock genes is tissue specific. Consistent with these latter observations, in the present study we confirm that surgical removal of the pineal gland has no effect on the diurnal rhythm of expression of PER2 in the rat SCN. More importantly, we found that pinealectomy has no effect on the diurnal rhythm of expression of PER2 in the BNST-OV, CEA and HIPP, indicating that the daily changes in expression of PER2 in these limbic forebrain structures are not dependent on endogenous rhythms of circulating melatonin. Interestingly, in spite of this lack of effect of melatonin on the rhythm of PER2 expression, the BNST, amygdala and HIPP have all been shown to contain melatonin binding sites [9, 15, 30] and to be responsive to melatonin. For example, melatonin has

been shown to modulate neural activity and serotonin levels in the amygdala [13, 16], to suppress complex partial seizure following amygdala kindling [12], to stimulate sexual activity following intra-amygdala infusion in impotent male rats [3] and to modulate estrogen receptor expression in the BNST [5]. Furthermore, melatonin has been shown to modulate potassium currents [6], kainite-induced proenkephalin and prodynorphin gene expression [32] and seizure susceptibility in the hippocampus [25]. The role of locally generated rhythms on the physiological or pharmacological effects of melatonin in these regions remains to be determined.

Our finding that pinealectomy had no effect on the diurnal rhythm of PER2 expression in the CP is of particular interest in light of a previous report of an essential role of the pineal gland in striatal Per1 mRNA and protein expression rhythms in mice [29]. This discrepancy may be related to species differences in the role of melatonin in the control of striatal clock gene expression or to differences in the sensitivity of striatal Per1 and Per2 genes to melatonin signaling. Consistent with this latter possibility, there is evidence that the regulation of Per1 and Per2 in different tissues involves different signaling mechanisms. Thus, different adrenergic signaling pathways have been implicated in the regulation of Per1 and Per2 in rat pineal [4, 27]. Furthermore, there is evidence that acute exposure to stress induces Per1 but not Per2 gene expression in the paraventricular nucleus of the hypothalamus and that Per1 but not Per2 genes contain glucocorticoid responsive element in their promoter region [26, 33]. Thus, it appears that Per1 and Per2 genes are differentially

regulated in different tissues; accordingly, it is possible that melatonin signaling is important for the expression of PER1 rhythms in the CP, as shown in mice, but plays no role in the expression of striatal PER2 rhythms as shown in the present study in the rat.

In summary, we find that the daily rhythm of PER2 expression in the BNST-OV, CEA, HIPP and CP in rats is unaffected by pinealectomy. Based on these finding, we conclude that the pineal hormone melatonin does not play a critical role in the regulation of the rhythmic expression of PER2 in these limbic forebrain structures in the rat.

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Figure caption.

Fig. 1

Left: Photomicrographs showing examples of PER2 expression in the SCN, BNSTov, CEA, HIPP and CP of sham-operated rats killed at ZT1 or ZT13 (magnification=x20). Right: graphs showing the mean (±s.e.m.) number of PER2-immunoreactive cells as a function of zeitgeber time (ZT) in each of these regions in sham-operated (SHAM, n=3/time point) and pinealectomized (PINX, n=3/time point) rats.

Figure