Research Article

Pineal melatonin deprivation alters the mRNA expression of melatonin and steroidogenic-related receptor genes in rat oviduct and uterus during the estrus stage of estrous cycle

Lia Alencar Coelho[1] *, Patricia Rodrigues Lourenço Gomes[1], Julieta Helena Scialfa[1], Carla C Maganhin[2], José Maria Soares Jr[2], Fernanda G Amaral[3], Russel J Reiter[4], José Cipolla Neto[1]

[1] Department of Physiology and Biophysics, Institute of Biomedical Sciences (ICB), University of São Paulo (USP), São Paulo-SP, Brazil

[2] Department of Obstetrics and Gynecology, Medical School, University of São Paulo, São Paulo-SP, Brazil

[3] Department of Physiology, Federal University of São Paulo, São Paulo-SP, Brazil

[4] Department of Cellular and Structural Biology, University of Texas Health Science Center, San Antonio, Texas, USA

*Correspondence: liac@usp.br, Tel: + 55 11 30917466.

Running title: Melatonin and steroidogenic-related receptors in uterus

Received: January 12, 2022; Accepted: April 5, 2022

ABSTRACT

The effects of pinealectomy and melatonin replacement therapy on the mRNA expression of melatonin (*Mt1*, *Mt2* and *Ror*α), steroidogenic (*Er*α - Estrogen α, *Er*β - Estrogen β, *Ar* - Androgen, *Pgr* - Progesterone, *Lhr* - LH and *Oxr* - Oxytocin receptors) -related receptors and melatonin-synthetic enzyme genes (*Tph1*, *Asmt* and *Aanat*) in oviduct and uterus of Wistar rats were studied. Tissues were collected from sham-pinealectomized (Control), pinealectomized (Pinx) and Pinx plus melatonin- supplemented (Pinx-Mel) females during the estrus stage of both the light (ZT6) and dark (ZT18) phases of the light-dark cycle. In oviduct, Pinx altered the mRNA expression of *Mt1*, *Mt2*, *Ror*α, *Er*α, *Pgr*, *Oxr*, *Asmt* and *Aanat* genes. In uterus, Pinx altered the mRNA expression of *Mt1*, *Mt2*, *Ror*α, *Lhr*, *Pgr*, *Oxr* and *Tph1* genes. Melatonin treatment partially or completely restored the *Mt2*, *Ror*α, *Er*α, *Oxr*, *Asmt* and *Aanat* mRNA expressions in oviduct and the *Mt1*, *Mt2* and *Ror*α mRNA expressions in uterus. These alterations varied according to the phase of light-dark cycle. The results suggest that pineal melatonin regulates the daily mRNA expression of melatonin and steroidogenic-related receptors in the rat oviduct and uterus during the estrus stage of estrous cycle. Melatonin also regulates the daily mRNA expression of *Asmt* and *Aanat* in oviduct and *Tph1* in uterus.

Key words: melatonin, pinealectomy; steroid, receptors, uterus, oviduct

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1. INTRODUCTION

Like proestrus, estrus is a stage which is part of the follicular phase of estrous cycle and is characterized by endocrine and molecular changes in female reproductive tract to support fertilization and subsequent embryo development before implantation. In rat, during this period, ovulation and oocyte fertilization take place and are controlled by several endocrine

and circadian signals (1). The ovulated cumulus-oocyte complexes are transported to the oviduct where fertilization occurs and then, the developing embryos are transported to the uterus for subsequent implantation (2). In addition to these reproductive hormones (LH, estradiol, progesterone, oxytocin) (2, 3), there is evidence that the expression (mRNA and/or protein) of their respective receptors in oviduct and uterus are involved in these events during the reproductive cycle (4-6).

Pineal melatonin plays an important role in circadian rhythmicity and in female reproductive events (7-9). Under *in vitro* conditions, melatonin affects positively the competence of the oocyte to develop into embryo after fertilization, (10, 11) by improving the fertilization and implantation rates (12-14). Melatonin is involved in controlling the timing of rat proestrus LH surge (15) and in modulating the human LH levels during follicular phase of the menstrual cycle (16). Melatonin regulates the expression of steroid receptors (androgen, estrogen, and progesterone) in oviduct and uterus at the time ovulation occurs (5), modulates myometrium function (17) and regulates progesterone production and LH receptor in luteal cells (18). Additionally, oocyte mitochondria synthetize melatonin documenting local melatonin synthesis (19) that is under control of pineal melatonin (20). All reproductive cells could have the potential to secrete melatonin (21) since melatonin synthetic enzymes (AANAT and ASMT) are expressed in ovary (20, 22) and uterus (23).

Melatonin protects oocytes from oxidative stress generated during the ovulation process (24); this protection is a result of the free radical scavenging and antioxidant actions of melatonin (25). In addition to these receptor-independent actions of melatonin, some of the effects of melatonin on female reproductive cycle are likely mediated by membrane melatonin receptors (Mt1 and Mt2), which are known to be present in most of reproductive-related cells and/or tissues (6, 17, 18, 20, 23, 26). These receptors belong to the G protein-coupled receptor superfamily and participate in the regulation of cAMP synthesis (27). There is also evidence that melatonin binds and activates members of the nuclear orphan receptor superfamily (28) including RORα whose mRNA is expressed in rat cumulus-oocyte complexes (20). Moreover, our previous research has demonstrated that the mRNA expression of these melatonin-related genes in ovary (20) and testes (29) are under control of circulating melatonin.

The present research will investigate the actions of pinealectomy (Pinx) and melatonin replacement on the mRNA expression of melatonin (*Mt1*, *Mt2* and *Ror*α) and steroidogenic (*Er*α - Estrogen α, *Er*β - Estrogen β, *Ar* - Androgen, *Pgr* - Progesterone, *Lhr* - LH and *Oxr* - Oxytocin receptors) -related receptor genes in oviduct and uterus of Wistar rats during the estrus stage of estrous cycle. We also will test whether mRNA expression of melatonin-synthetic enzyme genes (*Tph1*, *Asmt* and *Aanat*) is influenced by Pinx or melatonin treatment.

2. MATERIALS AND METHODS

2.1. Animals and ethics statement.

All the 36 adult (3-month-old, 200-220 g) Wistar females were housed on a 12L:12D light-dark cycle, in a temperature-controlled room (23°C ± 2°C), with food and water available ad libitum. The experimental procedures were conducted according to the guidelines of the Animal Care Committee of Ethics in Animal Experimentation of the Institute of Biomedical Sciences (CEUA 199/116f), University of São Paulo.

2.2. Experimental design.

The females were randomly divided into three groups (n = 6/group): sham-operated (Control), pinealectomized (Pinx) and pinealectomized melatonin-supplemented (Pinx-Mel).

The animals were euthanized by decapitation and the oviduct and uterine samples were collected during the light phase (six hours after lights on – zeitgeber time 6 - ZT6) or during the dark phase (six hours after lights off – zeitgeber time 18 - ZT18) of the 12h:12h light/dark cycle (22).

2.3. Estrous cycle evaluation and cytological analysis.

To evaluate the estrous cycle duration and stage, vaginal smears were collected daily for four consecutive weeks. These procedures were performed in two distinct periods: before the surgeries and immediately before the samples collection. Only females showing regular estrous cycle duration (4-5 days) were submitted to surgeries and the tissue samples were collected at estrus stage of the estrous cycle. The vaginal samples were processed according to Paccola et al. (30) description and stained by using the Harris-Schorr method.

2.4. Pinealectomy and melatonin replacement therapy.

After estrous cycle periodicity evaluation, anesthetized (intraperitoneal injection of ketamine and xylazine – 0.15 mL/100 g body weight) females were submitted to surgeries following Hoffman and Reiter (31) protocol with some modifications (29).

Melatonin (Sigma Aldrich, Saint Louis, MO, USA) solution was prepared according to the individual daily water intake and the body weight (1 mg/kg body weight) and daily added to drinking water only during the 12-hr of the dark phase of the light-dark illumination cycle (29). At the beginning of 12-hr light phase, the bottles were replaced by others with tap water free of melatonin. The melatonin treatment lasted three months.

2.5. RNA isolation and quantitative reverse transcriptase polymerase chain reaction (qRT-PCR).

Total RNA from oviduct and uterus samples previously frozen and stored at - 80ºC were isolated using guanidine isothiocyanate based reagent (TRIzol® Invitrogen, Thermo Fisher Scientific, Waltham, MA, USA) as instructed by manufacturer. First-strand cDNA was synthetized from 1µg of total RNA pellet using reverse transcriptase (Superscript III, Invitrogen, Thermo Fisher Scientific, Waltham, MA, USA) and qRT-PCR analysis was performed on QuantStudio 6 Flex Real-Time PCR equipment (Applied Biosystems, Inc, Foster City, CA, USA) with cDNA samples, SYBR green (Power SYBR green, ABI) and specific primers (Table 1). The relative analyses were calculated by $2^{-\Delta CT}$ method (32) and reported as arbitrary units. All measures were performed in duplicate for each sample and the *Rpl37a* was selected as reference gene according to our previous research (29).

2.6. Statistical analysis.

Data were expressed as mean ± SEM, calculated from at least five replications per group and analyzed in GraphPad Prism (GraphPad Software version 9.20; San Diego, CA, USA) using two-way ANOVA followed by Bonferroni´s post-test. The same data were analyzed in two different ways: first the pineal condition differences (Control, Pinx and Pinx-Mel) were analyzed within each phase of light-dark cycle and then, the phase differences (Light Phase and Dark Phase) were analyzed within each pineal condition group.

Table 1. List of qRT-PCR primers used in the present research.

Genes	Accession Number*	Primer sequence 5´- 3´	Base Pairs (bp)
Mt1	NM_053676.1	F (5'- 3'): CGTGGTGGACATCCTGGG R (5'-3'): CGAGGTCTGCCACAGCTAAACT	109
Mt2	NM_001100641.1	F (5'- 3'): TCCTCTCGGTGCTCAGGAAC R (5'-3'): AGGTCAGCCAAGGCCAGATT	75
Rorα	NM_001106834.1	F (5'- 3'): CGTGGTGGACATCCTGGG R (5'-3'): CGAGGTCTGCCACAGCTAAACT	93
Erα	NM_012689.1	F (5'- 3'): GGCATGATGAAAGGCGGGAT R (5'-3'): TCTTCGCAATCACCCAGACC	127
Erβ	NM_012754.1	F (5'- 3'): AAGTAGCCGGAAGCTGACAC R (5'-3'): TCTTCGCAATCACCCAGACC	68
Ar	NM_012502.1	F (5'- 3'): CCTATCCCAGTCCCAGTTGTG R (5'-3'): GGTACTGTCCAAACGCATGT	92
Pgr	NM_022847.1	F (5'- 3'): TGAAGCATCTGGCTGTCACT R (5'-3'):TAGTTATGCTGCCCTTCCATCG	91
Lhr	NM_012978.1	F (5'-3'): TTCCCAGGAGCAAGTAAGCC R (5'-3'): TAACGCTCTCGGTGGTATGG	84
Oxr	NM_021871.3	F (5'- 3'): TGAGTTGGCTAGAGCGTGTG R (5'-3'): CCTCTGATGGCTGAGTGACC	87
Tph1	NM_001100634	F (5'- 3'): CTCTTGGAGCTTCAGAGGAGAC R (5' - 3'): GACTCTCAGCTGCCCATCTTG	98
Asmt	NM_144759.2	F (5'- 3'): TGCCCGCACCCACTTCCTGTC R: (5'- 3'):GACCCGGGCAAGAATGAAGAG	112
Aanat	NM_012818	F (5'- 3'): AAAGTACACTCAGGCACCAATGT R (5' 3'):GGGAACATAGCTGCTTTATTAGTGTCAG	110
Rpl37a	NM_001108801	F (5'- 3'): TTGAAATCAGCCAGCACGC R (5'- 3'): TGCCAACGGCTCGTCTCT	74

*F: Forward, R: Reverse, * Accession Number is provided by the National Center for Biotechnology*

3. RESULTS

3.1. Effects of Pinx and melatonin replacement on melatonin-related receptor mRNA expression in oviduct and uterus.

The effects of Pinx and melatonin replacement on mRNA expression of melatonin-related receptors including *Mt1*, *Mt2* and *Rorα* in rat oviduct and uterus are shown in Figures 1 and 2. The results show that during the dark phase, Pinx decreased the mRNA expression of *Mt1* in oviduct (Figure 1A) and elevated the *Rorα* and *Mt2* mRNA expression in oviduct (Figure 1E) and uterus (Figure 1D), respectively; however, during the light phase, Pinx increased the mRNA expression of *Mt1* (Figure 1B) and *Rorα* (Figure 1F) in uterus. The melatonin treatment restored the mRNA profiles of *Rorα* in oviduct (Figure 1E), *Mt1* (Figure 1B) and *Mt2* (Figure 1F) in uterus. Relative to the phase differences, Pinx modified the daily mRNA expression pattern of *Mt1*, *Mt2* and *Rorα* in oviduct and uterus (Figure 2). Melatonin replacement restored mRNA expression of *Mt1* in uterus (Figure 2B), *Mt2* in oviduct (Figure 2C) and *Rorα* in oviduct (Figure 2E) and uterus (Figure 2F) to the control pattern.

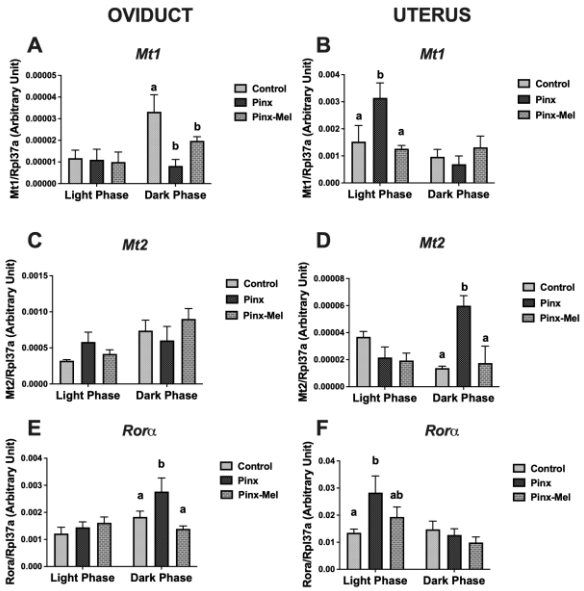

Fig. 1. Effects of Pinx and melatonin replacement on the mRNA expression of melatonin receptor genes in rat oviduct and uterus of rats at estrus stage during the light-dark cycle.

*Control: Sham-pinealectomized; Pinx: Pinealectomized; Pinx-Mel: Pinx melatonin-supplemented rats. Light Phase (ZT6: six hours after lights on), Dark Phase (ZT18: six hours after lights off). *P<0.05; **P<0.01 vs each phase of light-dark cycle. Reference gene: Rpl37a*

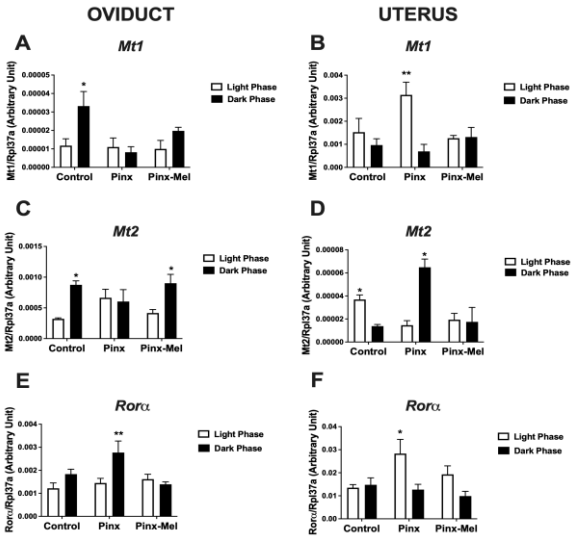

Fig. 2. Effects of Pinx and melatonin replacement on the mRNA expression of melatonin receptor genes in oviduct and uterus of rats at estrus stage during light-dark cycle.

*Control: Sham-pinealectomized rats; Pinx: Pinealectomized rats; Pinx-Mel: Pinealectomized melatonin-supplemented rats. Light Phase (ZT6: six hours after lights on) and Dark Phase (ZT18: six hours after lights off). * P<0.05; ** P<0.01) vs each pineal condition. Reference gene: Rpl37a.*

3.2. Effects of Pinx and melatonin replacement on steroidogenic-related receptor mRNA expression in oviduct.

The mRNA expression of steroidogenic (*Erα*, *Erβ*, *Ar*, *Pgr*, *Lhr* and *Oxr*) -related receptors in oviduct with/without pineal gland and phase differences were illustrated in Figures 3 and 4,

respectively. During the light phase, Pinx reduced mRNA expression of *Erα* (Figure 3A), *Pgr* (3D) and *Oxr* (3F), while melatonin treatment only restored the expression of *Oxr* to the control level. During dark phase, there was no significant effect of Pinx on most of the mRNA expression of steroidogenic-related receptor genes, except for the *Erα* which is upregulated by Pinx; melatonin treatment restored its expression back to level of control group (Figure 3A). Relative to phase differences, Pinx upregulated expression of *Erα* (Figure 4A) and *Oxr* (4F) genes during dark phase. In both cases, melatonin replacement restored to their expression to the levels of the control group.

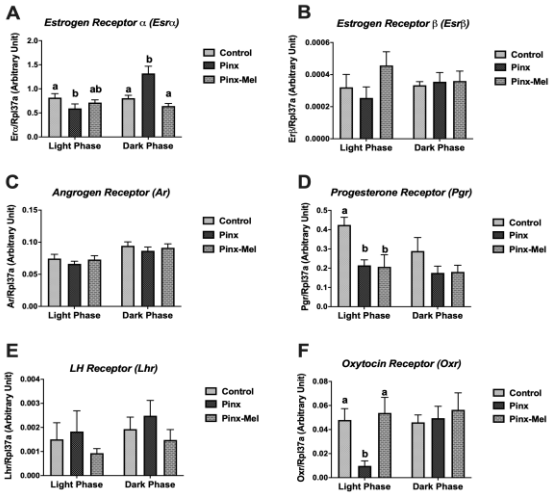

Fig. 3. Effects of Pinx and melatonin replacement on mRNA expression of steroidogenic-related receptor genes in rat oviduct at estrus stage during the light-dark cycle.

Control: Sham-pinealectomized rats; Pinx: Pinealectomized rats; Pinx-Mel: Pinx melatonin-supplemented rats. Light Phase (ZT6: six hours after lights on) and Dark Phase (ZT18: six hours after lights off). Different letters indicate P<0.05 vs each phase of light-dark cycle. Reference gene: Rpl37a

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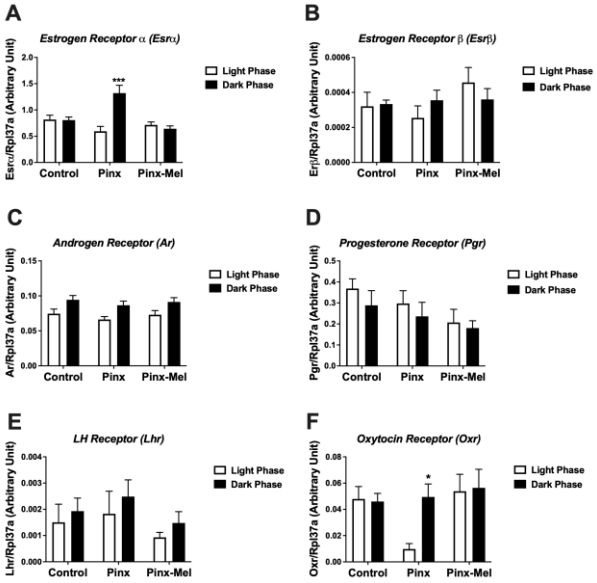

Fig. 4. Effects of Pinx and melatonin replacement on mRNA expression of steroidogenic related receptor genes in rat oviduct at estrus stage during the light-dark cycle.

*Control: Sham-pinealectomized; Pinx: Pinealectomized; Pinx-Mel: Pinx melatonin-supplemented rats. Light Phase (ZT6: six hours after lights on) and Dark Phase (ZT18: six hours after lights off). * P<0.05; *** P<0.01vs each pineal condition. Reference gene: Rpl37a.*

3.3. Effects of Pinx and melatonin replacement on steroidogenic-related receptor mRNA expression in uterus.

During light phase, Pinx decreased the mRNA expression of *Pgr* and *Oxr* genes (Figure 5F) and upregulated the *Lhr* gene in uterus (Figure 5E). Melatonin treatment had no significant effect on these parameters. In terms of the phase differences, Pinx only altered the mRNA expression of *Pgr* (Figure 6D) and *Lhr* (Figure 6E) genes and melatonin treatment failed to restore their expression to the levels of control group.

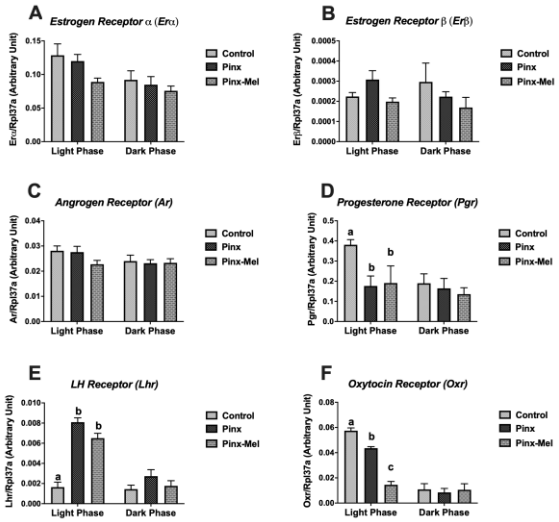

Fig. 5. Effects of Pinx and melatonin replacement on mRNA expression of steroidogenic-related receptor gene in rat uterus at estrus stage during light-dark cycle.

Control: Sham-pinealectomized rats; Pinx: Pinealectomized rats; Pinx-Mel: Pinealectomized melatonin-supplemented rats. Light Phase (ZT6: six hours after lights on) and Dark Phase (ZT18: six hours after lights off). Different letters above bars indicate P<0.05 vs each phase of light-dark cycle. Reference gene: Rpl37a.

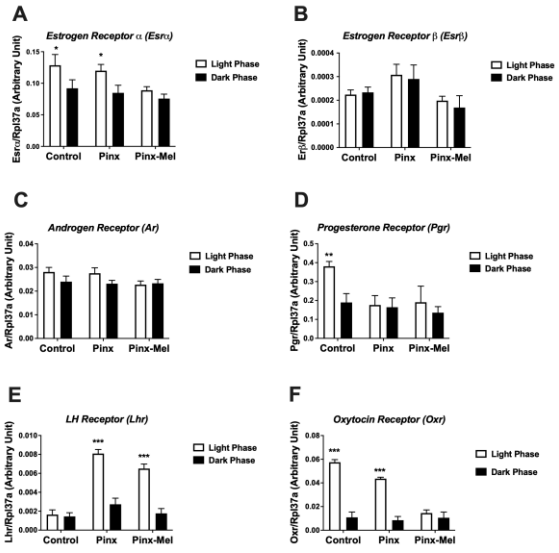

Fig. 6. Effects of Pinx and melatonin replacement on mRNA expression of steroidogenic related receptor gene in rat uterus at estrus stage during the light-dark cycle.

*Control: Sham-pinealectomized; Pinx: Pinealectomized rats; Pinx-Mel: Pinx melatonin-supplemented rats. Light Phase (ZT6: six hours after lights on) and Dark Phase (ZT18: six hours after lights off). * P<0.05; ** P<0.01vs each pineal condition. Reference gene: Rpl37a.*

3.4. Effects of Pinx and melatonin replacement on the mRNA expression of melatonin synthetic enzyme genes in rat oviduct and uterus.

Pinx altered the mRNA expression of only *Tph1* in uterus (Figure 7B). During the light and dark phases, there was an elevation in the levels of mRNA expression induced by the absence of circulating melatonin, but melatonin replacement therapy only restored the mRNA profile during the dark phase. Relative to the phase differences (Figure 8), pinealectomy altered the daily mRNA profile of *Asmt* (Figure 8C) and *Aanat* (Figure 8E) in oviduct. No effect of melatonin treatment was observed.

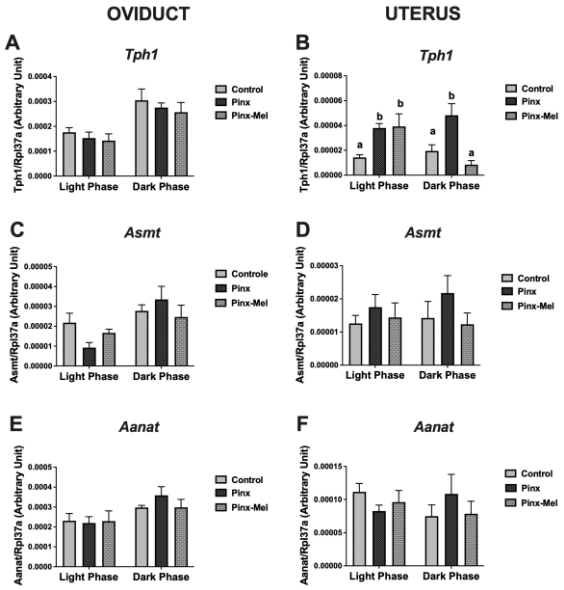

Fig. 7. Effects of Pinx and melatonin replacement on the mRNA expression of melatonin synthetic enzyme genes of rats at estrus stage during the light-dark cycle.

Control: Sham-pinealectomized rats; Pinx: Pinealectomized rats; Pinx-Mel: Pinealectomized melatonin-supplemented rats. Light Phase (ZT6: six hours after lights on) and Dark Phase (ZT18: six hours after lights off). Different letters above bars indicate P<0.05; P<0.01) vs each phase of light-dark cycle. Reference gene: Rpl37a.

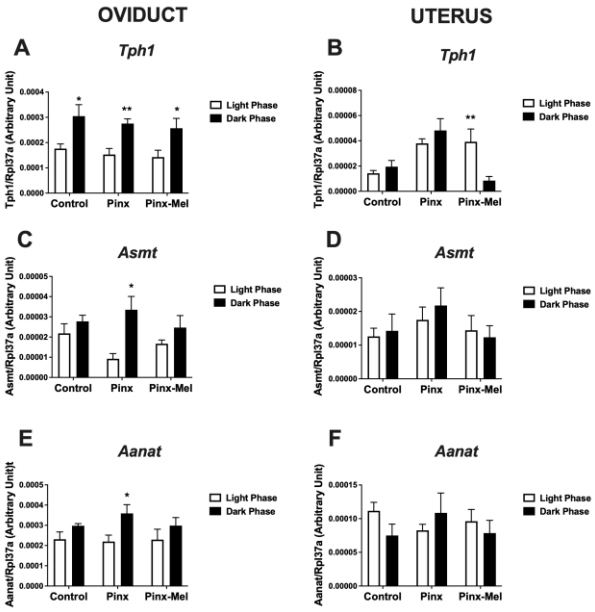

Fig. 8. Effects of Pinx and melatonin replacement on the mRNA expression of melatonin synthetic enzyme genes in oviduct and uterus of rats at estrus stage during the light or dark phases of light-dark cycle.

*Control: Sham-pinealectomized rats; Pinx: Pinealectomized rats; Pinx-Mel: Pinealectomized melatonin-supplemented rats. Light Phase (ZT6: six hours after lights on) and Dark Phase (ZT18: six hours after lights off). * P<0.05; ** P<0.05vs each pineal condition. Reference gene: Rpl37a.*

4. DISCUSSION

This research investigated the effects of the absence of circulating melatonin and nocturnal melatonin replacement on the gene expressions of melatonin-related receptors (*Mt1*, *Mt2* and *Ror*α), steroidogenic-related receptors (*Er*α, *Er*β, *Ar*, *Pgr*, *Lhr* and *Oxr*) and melatonin synthetic enzymes (*Tph1*, *Asmt* and *Aanat*) in oviduct and uterus of rats during estrus stage of estrous cycle.

The membrane receptor-mediated actions of melatonin during the follicular phase of estrous cycle are well documented. Melatonin receptor 1 (MT1) is responsible for regulation of ovarian follicular apoptosis (33, 34), oocyte maturation (35) and luteinization process (36). Evidence shows that both MT1 and melatonin receptor 2 (MT2) work jointly to modulate ovarian cells during follicle development (37, 38). Moreover, the effect of melatonin on oocyte maturation (39) and steroidogenesis (40) may be also mediated by MT2. The binding affinity for MT1 and MT2 in ovary varies according to the stage of estrous cycle (26) and their mRNA expression levels are regulated by melatonin (34, 37, 40). Although the role of melatonin in oviduct and uterus via its receptors is still unclear, it has been demonstrated that at estrus stage, the expression of MT1 receptor was upregulated by melatonin in uterus but not in oviduct (6). Additionally, it was also observed that the proliferative activity of rat uterine cells is inhibited by melatonin via MT1 activation (41).

The present findings demonstrated that the absence of pineal melatonin by Pinx modified the daily mRNA expression profiles of melatonin-related receptors (*Mt1*, *Mt2* and *Ror*α) in the rat oviduct and uterus during the estrus stage. Removal of the pineal increased *Mt1*, *Mt2* and *Ror*α mRNA expression in uterus and decreased *Mt1* and increased *Ror*α mRNA expression in oviduct. Variations in the mRNA expression of these receptors were dependent on the phase of light-dark cycle. It has been demonstrated that the daily changes in liver mRNA and protein expressions of MT1, MT2 and RORα exhibit a circadian rhythmic profile which is under control of circulating melatonin (42). In our previous study, we found that the mRNA expression of these receptors in ovarian cells varied according to maturational stage of cumulus-oocyte complex (COC) during the follicle development which was also under control of circulating melatonin (20). It seems that the daily mRNA variations of these receptors in reproductive tissues regulated by pineal melatonin is influenced by the stage of estrous cycle. It has already been documented that the estrous cycle drives the circadian rhythmicity of the clock gene expression in rat ovary and uterus and the steroid hormones participate in this regulatory mechanism (43). During the follicular phase of rat estrous cycle, the high blood levels of estradiol promote the ovulatory LH surge (3) which occurs on afternoon of proestrus and is controlled by suprachiasmatic nucleus (SCN) signaling (1) and pineal melatonin (15). During estrus stage, estradiol and progesterone are responsible for mating behavior and morphological changes in uterus (44) and, both steroid hormone levels (45) and uterine morphology (46) are altered by pinealectomy. It has been also demonstrated that estradiol regulates the melatonin receptors in oviduct (47) and uterus (48) through estrogen receptor pathway (47).

Considering the daily mRNA expression of steroidogenic-related receptors (*Erα*, *Erβ*, *Ar*, *Pgr*, *Lhr* and *Oxr*) in oviduct and uterus, our study demonstrated that Pinx altered the *Erα* expression in oviduct by reducing the mRNA levels during the light phase and augmenting during the dark phase. The significant influence of circulating melatonin *on Erα* mRNA expression in uterus or *Erβ* mRNA expression in oviduct and uterus were not observed. During the light phase, Pinx decreased the mRNA expression of *Pgr* and *Oxr* genes in oviduct and uterus and increased the *Lhr* mRNA expression in uterus. It is already documented the involvement of estrogen receptors (ERα and ERβ) in oviduct (49) and uterus (50, 51) functions during the reproductive cycle. The expression levels of these two isoforms of estrogen receptors in oviduct and uterus varied according to the stage of rat estrous cycle with a predominant cycled mRNA expression of *Erα* isoform (52). It has been also demonstrated the mRNA expression of *Erα* and *Pgr* genes in bovine oviduct is predominant during the follicular phase of estrous cycle (53). Likewise, the cycled variation of mRNA expression of oxytocin receptor in the ovine oviduct (54) and in the rat uterus with increased mRNA levels during the follicular phase regulated by steroid hormones (55) suggest an interaction among these receptors in mediating the oviduct and uterus functions during the estrous cycle that could be controlled by melatonin. This melatonin action involves reduction of the oxytocin-induced uterine prostaglandin release (56) through oxytocin receptors which are regulated by steroid hormones (57) and inhibition of oxytocin-induced uterine contractions in pregnant and non-pregnant females (58). Moreover, although the physiological role of LH receptor in uterus is still unclear, it seems that the mRNA expression of LH receptor in rat uterus is responsible for mediating the LH action on the reduction of uterine contractions at ovulation (59) and the disruption of LH receptor gene (LH receptor knockout mice) also altered the expression of *ERβ* and *PGR* in uterus (60). Genetic polymorphism, disruption and/or anormal pattern of mRNA expression of *Erα* (61, 62), *Erβ* (63, 64), and *Pgr* (65) genes in uterine cells has been associated to endometrial cancer (61-63) and/or endometriosis (64, 65). The regulation of the mRNA expression of these receptors by melatonin in uterus observed in our study suggests that melatonin plays an important role as a therapeutic agent in steroid hormone-dependent uterine disorders. Beneficial effects of melatonin on endometrial cancer (66) whose actions are mediated by MT1 receptor have been demonstrated (67). Similarly, melatonin also plays an important beneficial role in inhibiting the estrogen-induced migration, invasion, and epithelial-mesenchymal transition in normal and endometriotic endometrial cells (68) through its anti-inflammatory, immunoregulatory and antioxidant properties (69).

Our results also show that the effects of pineal melatonin on the modulation of mRNA expression of these receptors in rat oviduct and uterus depends on the light-dark cycle. As described in our previous report (22), the tissue samples were selectively collected at two time points of light-dark cycle (one at the light phase and another at the dark phase) which were statistically analyzed in two means: we first investigated the effects of Pinx or Pinx-Mel on mRNA expression at the middle point of each phase of light-dark cycle and then, we studied the mRNA expression of phase differences in Control, Pinx and Pinx-Mel animals. In both statistical analyses, Pinx affected the *Mt1* (Figures 1A, 1B, 2A and 2B) and *Rorα* (Figures 1E, 1F, 2E and 2F) mRNA expression in oviduct and uterus, *Erα* (Figures 3A and 4A) and *Oxr* (Figures 3F and 4F) mRNA expression in oviduct and *Mt2* (Figures 1D and 2D), *Pgr* (Figures 5D and 6D) and *Lhr* (Figure 5E and 6E) mRNA expression in uterus. Considering only the pineal condition differences, Pinx altered the mRNA expression of *Pgr* (Figure 3D) in oviduct and *Oxr* (Figure 5F) and *Tph1* (Figure 7B) in uterus whereas specific phase differences in Pinx animals were observed in mRNA expression of *Mt2* (Figure 2C), *Asmt* (Figure 8C) and *Aanat* (Figure 8E) in oviduct. These results confirm the circadian profile of mRNA expression of these receptors was altered by circulating melatonin, but it should be stated that the putative

lack of effects of Pinx on the mRNA expression of a given receptor in the oviduct or uterus (for example, *Erα* mRNA expression in uterus) does not mean that circulating melatonin has no effect on this daily expression profile but that the significant differences may be seen on another time point (different from ZT 6 and ZT 18) of the light-dark cycle.

The present study also shows that Pinx altered the mRNA levels of some melatonin-synthetic enzymes in rat oviduct (*Asmt* and *Aanat*) and/or uterus (*Tph1*) similar to the influence of circulating pineal melatonin on local peripheral melatonin synthesis as evaluated by the daily mRNA expression profile of these enzymes in the ovary (20) and testis (29) as demonstrated in our previous studies. However, although all cells have the potential to synthetize melatonin (19, 21), it is unlikely that oviductal and uterine cells are secreting melatonin during the estrus stage of estrous cycle since no AANAT protein expression in oviduct or uterus is detected in our study (unpublished data).

Our findings provide the information that melatonin replacement partially or completely restores the daily mRNA expression pattern of most genes studied. This effect of melatonin might be mediated by the well-known chronobiotic effect of melatonin on regulating circadian rhythms as well as might be dependent on the nocturnal immediate and diurnal prospective effects of melatonin discussed by Cipolla-Neto and Amaral (70). In addition, it should be considered that the effects of melatonin replacement would depend on several other factors including way of administration of melatonin treatment, melatonin resulting plasmatic concentration and the duration of the resulting nocturnal melatonin profile (71, 72).

In conclusion, circulating melatonin regulates the daily mRNA expression of melatonin receptors, steroidogenic-related receptors and melatonin synthetic enzymes in rat oviduct and uterus during the estrus stage of estrous cycle. These results suggest the potential use of melatonin in uterine disorders associated to steroid receptors.

ACKNOWLEDGMENTS

This research was funded by Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP), São Paulo, Brazil, grant number FAPESP 2019/24327-5.

AUTHORSHIP

LC conceived and designed the research and, wrote the paper. PG, CM, JS and FA collected the data and performed the experiments. JSJr analyzed data and RR wrote and revised the paper. JC-N revised the paper and was responsible for funding acquisition.

CONFLICT OF INTEREST

We declare there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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Please cite this paper as:
Coelho, L.A., Gomes, P.R., Scialfa, J.H., Maganhin, C.C., Soares Jr., J.M., Amaral, F.G., Reiter, R. and Cipolla-Neto, J. 2022. Pineal melatonin deprivation alters the mRNA expression of melatonin and steroidogenic-related receptor genes in rat oviduct and uterus during the estrus stage of estrous cycle. Melatonin Research. 5, 1 (Apr. 2022), 68-83. DOI:https://doi.org/https://doi.org/10.32794/mr112500121.