Circadian clock regulation of melatonin MTNR1B receptor expression in human myometrial smooth muscle cells

Stephen Beesley, Justin Lee, and James Olcese*

Department of Biomedical Sciences, and Program in Neuroscience, Florida State University College of Medicine, 1115 West Call Street, Tallahassee, FL 32306, USA

*Correspondence address. E-mail: james.olcese@med.fsu.edu

Submitted on February 1, 2015; resubmitted on April 17, 2015; accepted on April 21, 2015

ABSTRACT: Circadian genes are expressed in virtually all cells and tissues, and circadian rhythms influence many bodily processes, including reproductive physiology. The expression of hMTNR1B is suppressed during pregnancy until late in term (much like the oxytocin receptor), at which time it is up-regulated to allow for the nocturnal melatonin/oxytocin synergy, which promotes strong nocturnal contractions. Little is currently known about the regulation of hMTNR1B, nor about its functional significance in the myometrium. We, therefore, aimed to elucidate some of the transcription factors that regulate hMTNR1b gene expression in the human myometrium and to determine if hMTNR1b is under circadian control. In this study, we used immortalized and primary myometrial cells that were assessed for circadian gene expression rhythms using real-time bioluminometry and quantitative PCR. Chromatin immunoprecipitation examined the binding of the clock gene product brain and muscle aryl hydrocarbon receptor nuclear translocator (ARNT)-like protein 1 (BMAL1) to the promoter of the hMTNR1B gene. Overexpression studies tested the role of circadian locomotor output cycles kaput (CLOCK) and its partner BMAL1 in regulating hMTNR1B expression. We confirmed circadian clock gene expression in both immortalized human myometrial cells and primary myometrial cell cultures. We further showed that the hBMAL1 protein binds to an E-box motif in the proximal promoter of the hMTNR1B gene. Overexpression studies demonstrated that the BMAL1/CLOCK complex activates expression of hMTNR1B leading to a circadian rhythm in phase with the E-box driven clock gene hPER2 (Period 2). These results indicate, for the first time, the presence of a functional circadian clock in the human myometrium with the hMTNR1B gene as a clock controlled target. Further investigations could open new vistas for understanding the regulation of uterine contractions and the timing of human labor.

Key words: circadian regulation / MTNR1B / melatonin / myometrium / labor

Introduction

According to the Institute of Medicine of the National Academies of Sciences (http://national-academies.org), the economic burden of preterm births in the United States is well over US$ 62 billion per year (>US$ 100 000 per infant). Despite this continually increasing medical challenge, there has been relatively little progress in the past 20 years in understanding the processes initiating labor, whether term or preterm. In Western societies, preterm labor occurs in more than 12% of all pregnancies. It remains the major cause of perinatal morbidity and is associated with 70% of neonatal mortality (Smith, 2007).

There is general consensus that many of the genes involved in labor activation are directly or indirectly involved in the inflammatory cascade (Bollapragada et al., 2009), which can be recruited either by natural labor-associated pathways, or by acute inflammation or stress (Smith, 2007). However, gene regulation in the pregnant term myometrium may well involve other processes as well, including smooth muscle stretch (Sooranna et al., 2007), functional progesterone withdrawal (Smith, 2007) and cell signaling pathways leading to increased membrane potential and propagation of action potentials (Smith, 2007). Recently, we published evidence for a novel hormonal signal that may play an important role in the circadian timing of human parturition, opening new avenues for understanding the regulation of human labor. Specifically, melatonin type 2 receptor (MTNR1B) signaling in the human myometrium results in dramatic synergism with oxytocin receptor signaling (Sharkey et al., 2009, 2010), which may provide a critical hormonal trigger for the initiation of labor (Olcese, 2012). Of importance in this regard is that expression of both MTNR1B melatonin and oxytocin receptors in myometrium is suppressed during most of normal term pregnancy, only to reappear at the time of labor (Sharkey et al., 2009).

Given that melatonin is recognized as a major output signal of the brain’s circadian clock to modulate peripheral cellular clocks via specific G-protein-coupled membrane receptors (Dubocovich et al., 2010), we hypothesized that MTNR1B melatonin receptor expression may also be under circadian regulation in the human myometrium. Recent studies have demonstrated the expression of circadian clock genes in the
rodent uterus (Ratajczak et al., 2010; Isayama et al., 2015), consistent with the notion of peripheral 24-h clock mechanisms involving transcriptional/translational feedback loops involving the rain and muscle ary hydrocarbon receptor nuclear translocator (ARNT)-like protein 1 (BMAL1), circadian locomotor output cycles kaput (CLOCK), PER1/2 and CRY1/2 proteins that control the circadian expression of key target genes (Gustafson and Partch, 2014).

In this study, we demonstrate for the first time that human myometrial smooth muscle cells express key clock genes in a circadian manner. Additionally, we show that the human myometrial melatonin MTNR1B receptor (the dominant MTNR form in this tissue; Sharkey et al., 2009; Olcese et al., 2013) undergoes a 24-h mRNA expression rhythm and this expression is under the control of the clock transcription factors, CLOCK and BMAL1. These findings provide new insight into cellular mechanisms modulating receptor expression in the human myometrium and present potential pharmacological agents to maintain pregnancy in the face of preterm labor.

Materials and Methods

Cell culture and real-time bioluminometry

All cells were maintained at 37°C in a humidified atmosphere with 5% CO2. Immortalized hTERT myometrial cells (Sharkey et al., 2009) were cultured in phenol-red free medium (MediaTech), supplemented with 10% FBS (Gibco), 25 mM HEPES (Sigma), 4 mM L-glutamine (Sigma) and 1% amphotericin B/penicillin/streptomycin (Gold Biotechnology) (Yamazaki and Takahashi, 2005). Dishes were sealed with a cover slip and placed on a Lumicycle™ (Actimetrics) for long-term recording.

Quantitative PCR

Approximately 1 × 10⁶ hTERT/primary pregnant or non-pregnant cells were synchronized with 50% horse serum for 2 h, 24 h prior to RNA isolation. RNA was collected every 4 h for 20 h (6 time points), using RNeasy™ kit (Qiagen). Total RNA was converted to cDNA (Script™ cDNA synthesis kit, Biorad). Transcripts were amplified using Sybr Green qPCR mastermix (Quanta Biologicals) on the iCycler™ (Biorad). The normalization control was h18S RNA. This housekeeping gene is ideal for these experiments as its expression does not itself change over 24 h. Relative transcript quantification was achieved using a standard curve generated for each primer set. The primer sequences are shown in Table III.

Overexpression

The hMTNR1B::luc reporter was constructed using a 940 bp fragment of the MTNR1B (MT2) promoter 5′ to the transcriptional start site (TSS). The primers were: F1 cgccgctagcgaagagagcctgctgctgctgcat and R1 cgccgatatccgttctctgccatctgcagcagt. The resulting DNA was cloned into the same modified pGL4.16::luc vector, as described above, using NheI and EcoRV (5′–3′).

Table I Background data for tissue samples from non-pregnant women.

<table>
<thead>
<tr>
<th>Patient ID</th>
<th>Age</th>
<th>Number of pregnancies</th>
<th>Number of caesarean sections</th>
</tr>
</thead>
<tbody>
<tr>
<td>5935</td>
<td>44</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>5934</td>
<td>40</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>5495</td>
<td>59</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>5791*</td>
<td>48</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>5843*</td>
<td>39</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>5335*</td>
<td>67</td>
<td>4</td>
<td>0</td>
</tr>
</tbody>
</table>

*Cells used for overexpression studies.

Table II Background data for tissue samples from pregnant women.

<table>
<thead>
<tr>
<th>Patient ID</th>
<th>Age</th>
<th>Number of weeks pregnant</th>
<th>Number of pregnancies</th>
<th>Number of caesarean sections</th>
</tr>
</thead>
<tbody>
<tr>
<td>9929</td>
<td>31</td>
<td>39</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>9950</td>
<td>19</td>
<td>39</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>9951</td>
<td>28</td>
<td>39</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>5945</td>
<td>18</td>
<td>39</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>9930</td>
<td>24</td>
<td>39</td>
<td>2</td>
<td>2</td>
</tr>
</tbody>
</table>

Table III Primer sequences.

<table>
<thead>
<tr>
<th>Gene</th>
<th>F1 200 nM</th>
<th>R1 200 nM</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>hiPer2</td>
<td>TAC GCT GGC CAC CTT GAA GTA</td>
<td>CAC ATC GTG ACG GGC CAG GA</td>
<td>Krugluger et al. (2007)</td>
</tr>
<tr>
<td>hBMAL1</td>
<td>GTA AC TCA GCT GCC TGC TC</td>
<td>TAG CTG TTG CCC TCT CGT CT</td>
<td></td>
</tr>
<tr>
<td>hMNTR1B</td>
<td>TTG TGA TCT TTG CCA TCT GCT GGG</td>
<td>GCT GCC CTT GGA AGC ATC TTG AAT</td>
<td></td>
</tr>
<tr>
<td>h18S RNA</td>
<td>ATG GCC GTT CTT AGT TGG TG</td>
<td>CGC TGA GCC AGT CAG TGT AG</td>
<td>Pont et al. (2012)</td>
</tr>
</tbody>
</table>
Approximately, $0.3 \times 10^6$ hTERT or non-pregnant primary cells (from donors #5335, #5791 and #5843) were transfected with 300 ng of the respective construct, made up to 1000 ng with pCDNA3.1, using Mirus transfection reagent (Mirus Biologicals). After 48 h, RNA was isolated, reverse transcribed to cDNA and quantified, as above.

**Chromatin immunoprecipitation**

Chromatin immunoprecipitation (ChIP) was performed using the ChIP-IT Express™ kit (Active Motif), according to the manufacturer’s instructions. The guinea pig anti-hBMAL1 antibody (1:50) was a kind gift from Dr Choogon Lee (Florida State University). The MTNR1B promoter-specific primers were: FW- CAGTGTATGAGGGTTCTGGTTGCT, and REV-GGGTTAGGACAAGAAGAAGATTGAGGC, which amplified a 234 bp region between $-1026$ and $-793$ 5′ to the TSS, which contains an E-box motif (CACNTG) between $-848$ and $-843$ bp upstream of the TSS. As a negative control against non-specific binding, an anti-rabbit goat secondary antibody was also added at a 1:1000 concentration to a sample (as guinea pig serum was not available). An additional negative control lacking antibodies was also used.

**Statistical analysis**

Statistical analysis was carried out using Graphpad Prism software. Data were analyzed using a one-way ANOVA with a Bonferroni post-hoc correction. All hTERT data are derived from three independent repeats and all data from primary myometrial cells are from three to five individual donors. Real-time bioluminescence data were fitted to a damped sine wave using the Lumicycle® Analysis Software (Actimetrics).

**Results**

From our initial studies and those of others (Glattre and Bjerkedal, 1983; Cooperstock et al., 1987; Panduro-Baron et al., 1994), we hypothesized that there is a strong circadian component coordinating the onset of parturition. To investigate this hypothesis, we developed an immortalized myometrial cell line (hTERT cells) with stably integrated circadian reporter constructs to accurately report circadian data in real-time. Further, we transiently transfected primary myometrial cells for short-term analyses of circadian rhythmicity.

**Circadian rhythmicity of human myometrial cells**

As a first step in the characterization of circadian rhythmicity in human myometrial smooth muscle cells, we determined the endogenous expression of two clock genes, BMAL1 and PER2, which represent key

![Figure 1](https://academic.oup.com/molehr/article-abstract/21/8/662/1471512/1)
components of the positive and negative limbs of the accepted circadian clockwork model (Lowrey and Takahashi, 2011). Cultured primary myometrial cells from both non-pregnant (NP) and pregnant (P) donors clearly expressed BMAL1 and PER2 (Fig. 1A). Real-time bioluminometry experiments were then conducted with immortalized human myometrial smooth muscle cells (hTERT) following stable transfection with constructs comprised of the promoter sequences of either mBmal1 or mPer2 linked to the luciferase reporter (Fig. 1B). Significant in vitro oscillations of both reporter constructs were noted for over five cycles and were clearly antiphasic to one another. Similar circadian oscillations of mBmal1: luc were also detected after transient transfection of primary myocytes from pregnant donors (Fig. 1D). Interestingly, in contrast to reliable circadian oscillations in cells from pregnant donors, bioluminometry results were inconsistent in primary cells from non-pregnant donors (Fig. 1C), suggesting that circadian rhythmicity is more pronounced or persistent in culture in the myometrium during pregnancy. Alternatively, patient age or pathology of the myometrium at the time of hysterectomy (leiomyomas, adenomyosis) may disrupt circadian rhythmicity in non-pregnant myometrial cells in culture.

To confirm further endogenous rhythmicity of BMAL1 and PER2 expression, cultured hTERT cells were synchronized, then collected every 4 h for 24 h. RNA was isolated at each time point and expression of hBmal1 and hPer2 were quantified using qPCR. Additionally, expression of hMTNR1B was also quantified as a function of time. As found with RT-bioluminescence (Fig. 1B), hBMAL1 and hPER2 oscillations were significant and antiphasic (Fig. 2A and B). Although less dramatic, a clearly circadian expression rhythm was also seen with MTNR1B (Fig. 2C) and it was also antiphasic to BMAL1, i.e. it resembled the hPER2 rhythm in terms of phase.

Phase relationship between MTNR1B and PER2

In view of the above findings, we then chose to determine the phase relationship between MTNR1B and PER2 in primary myometrial smooth muscle cells from non-pregnant and pregnant donors. Figure 3 demonstrates a good correlation of phase in two of three independent experiments with primary cells from non-pregnant donors. In contrast, expression rhythms of PER2 and/or MTNR1B were only seen in some of the experiments with myometrial cells from pregnant donors (Fig. 4), with only occasional phase correlation. However comparison of the crossing thresholds from the qPCR experiments found no statistical differences between the data from pregnant and non-pregnant donors regardless of the gene analyzed (MTNR1B: NP = 30.63 ± 1.79 versus P = 31.88 ± 2.13; Per2: NP = 27.20 ± 0.83 versus P = 27.70 ± 1.44).

Regulation of hMTNR1B expression in myometrial cells

As the antiphasic relationship of MTNR1B and BMAL1 expression is suggestive of potential regulation of MTNR1B expression by the BMAL1-CLOCK positive limb of a circadian clock, it seemed important to

**Figure 2.** Circadian oscillation of hMNTR1B melatonin receptor expression in hTERT cells. Cultured hTERT cells were synchronized by serum shock, then harvested over the circadian day. Transcript levels for hPer2 (A), hBmal1 (B) and hMNTR1B (C) all show a robust rhythm over the 24 h time course (hPer2 and hBmal1 are used here as positive circadian controls). Statistical analysis was done by a one-way ANOVA with Bonferroni post-hoc correction. The error bars represent the SEM of three replicate experiments. *P < 0.05 versus CT12.
determine whether the BMAL1 protein binds to the hMTNR1B promoter, particularly as the latter contains a non-canonical E-box in the proximal 1 kb (Fig. 5A). ChIP was conducted with nuclear extracts of the TERT cells using a specific BMAL1 antibody and PCR primers encompassing the E-box motif of the MTNR1B promoter. ChIP analysis revealed binding of endogenous BMAL1 to this E-box motif upstream of the coding region for MTNR1B. Signals were absent in extracts treated without the primary antibody or with a non-specific anti-rabbit antibody, suggesting specific binding of this clock protein to the promoter.

Additional evidence for the functionality of the non-canonical E-box in the MTNR1B promoter was obtained by acute overexpression of both BMAL1 and/or CLOCK proteins in both hTERT cells and in primary myometrial smooth muscle cells from a non-pregnant donor. In both cell systems, combined overexpression of BMAL1 and CLOCK led to significant induction of the MTNR1B promoter (Fig. 5C and D).

Discussion

The two known mammalian melatonin receptors (MTNRs) were cloned in the mid-1990s (Reppert et al., 1994, 1995, 1996) and subsequently shown to be unique G-protein-coupled membrane receptors based on their molecular structure and chromosomal localization (cf. Dubocovich et al., 2010). Both receptors signal via multiple intracellular pathways, including inhibition of cyclic AMP and cyclic GMP, activation of protein kinase C (PKC) and extracellular signal-regulated protein kinase (ERK1/2), and many other pathways (cf. Dubocovich et al., 2010).

However, little is known regarding the regulation of melatonin receptors. Several years ago, Johnston and coworkers evaluated the control of the MTNR1a receptor in the rat pituitary gland, whose expression is high in the neonate as a consequence of stimulation by the transcription factor pituitary homeobox-1 (PITX-1), but whose expression declines dramatically with the onset of puberty (Johnston et al., 2003, 2006a, b). Recently, this group also reported that the MTNR1a regulation is independent of gonadotrophin-releasing hormone-stimulated early growth response factor-1 (EGR-1) expression (Bae et al., 2014).

Similar analyses have rarely been undertaken in the human thus far, presumably due to the lack of any report demonstrating up- or down-regulation in any human tissue. Melatonin MT1 and MT2 receptors (MTNR1A and MTNR1B) are expressed in a variety of human tissues, including the human myometrium (Schlabritz-Loutsevitch et al., 2003). Work in our lab has demonstrated that these receptors are functional and, through PKC, ERK1/2 and myosin light chain kinase signaling, have pro-contractile effects, particularly in the presence of oxytocin (Sharkey et al., 2009, 2010). Analogous to the regulation of oxytocin receptors, the expression of melatonin receptors in the human myometrium appears to be repressed during normal pregnancy, but in late term labor...
its expression is dramatically elevated (Sharkey et al., 2009). The goal of the current studies was to begin the analysis of transcription factors that up-regulate the \( MTNR1B \) melatonin receptor in the human myometrium. Along these lines, we recently demonstrated that the expression of both NFATc1 (nuclear factor of activated T-cells, cytoplasmic 1) and the \( MTNR1B \) are rhythmic and antiphasic to one another in cultured human immortalized myometrial smooth muscle cells (Olcese and Beesley, 2014).

Using a bioinformatics approach, we have identified a number of relatively common control elements within the proximal 2 kb of the \( hMTNRs \). These include enhancer elements (E-boxes) for the circadian transcription factors BMAL1 and CLOCK. In mammals, circadian rhythms are generated by transcriptional/translational feedback loops with a built-in time delay of feedback inhibition (Shearman et al., 2000). CLOCK, its paralog NPAS2 (Neuronal PAS domain-containing protein 2), and BMAL-1 are basic helix-loop-helix transcription factors in the positive limb of the clock that directly induce transcription of the Period (Per) and Cryptochrome (Cry) genes, which themselves are the major components of the negative limb (Partch et al., 2014).

**Figure 4** Circadian oscillation of \( hMNTR1B \) expression in pregnant human myometrial cells. Primary myometrial cells were cultured from five independent pregnant donors. Cells were subsequently synchronized and harvested over the circadian day. Expression levels for \( hPer2 \) (black) and \( hMNTR1B \) (red) are shown for each donor.
As a starting point for our cellular investigations, we were able to document the expression of hBMAL1 and hPER2 in myometrial biopsies from both non-pregnant and pregnant women (Fig. 1A). Additionally, the presence of endogenous Bmal1::luc and Per2::luc rhythms in immortalized human myometrial smooth muscle cells could also be identified (Fig. 1B). As would be predicted of a functional self-sustained circadian clock, the Per2/luc rhythm was 12 h antiphasic to that of Bmal1/luc following serum shock. Subsequent assessment of these cells (Fig. 2A and B) confirmed rhythmic and antiphasic expression of endogenous hPER2 and hBMAL1 transcripts. In addition, rhythmic hMTNR1B expression could be detected in these cells, with a phase matching that of hPer2 (Fig. 2C). This is consistent with the hypothesis that E-boxes in the promoter regions of both hPER2 and hMTNR1B genes are regulated by BMAL1/CLOCK binding. This also conforms to reports in mice that demonstrated 24-h variations in hypothalamic melatonin receptor expression (Masana et al., 2000; Odo et al., 2014).

To explore this hypothesis further, we assessed the phase relationship of hPER2 and hMTNR1B expression in cultured primary human myometrial smooth muscle cells from both non-pregnant and pregnant women donors (Figs 3 and 4). Despite substantial inter-individual variations, peak and trough time-points for hPER2 and hMTNR1B expression were generally (for 2 of 3) in phase for cells derived from non-pregnant women (Fig. 3), similar to our hTERT results (Fig. 2). Although rhythmic variations in the expression patterns of both genes were also detected in cells from pregnant donors (Fig. 4), the correlations between the hPER2 and hMTNR1B transcript rhythms were less obvious. This is similar to a previous report by Ratajczak et al. (2010) using pregnant mice uteri, wherein the rhythmic expression of mPer2 transcripts was also surprisingly erratic. We can offer no reasonable explanation for these differences at present apart from the obvious differences in endocrine status. Subsequent data on myometrial clock gene expression at the protein level may help to resolve these differences.

To validate BMAL1 binding to the consensus E-box at −840 bp upstream of the proximal hMTNR1B promoter start site, we performed ChIP using immortalized human myometrial cells (hTERT). Clear signals were seen in the input and ChIPed samples (Fig. 5A), with no signals in the control samples. To expand on the ChIP results, we constructed an hMNTR1B/luc reporter (Fig. 5B). Upon co-expression with BMAL1 together with its dimerization partner, CLOCK, we could show significant up-regulation of the hMNTR1B::luc reporter in both immortalized hTERT cells (Fig. 5C) and primary human myometrial cells (Fig. 5D). Indeed, in the case of primary myometrial cells, overexpression of CLOCK alone resulted in significant activation of the hMTNR1B promoter construct, suggestive of a substantial level of endogenous BMAL1 protein in primary myometrial cells, perhaps even more than presumed to be expressed in the immortalized hTERT cells as inferred from the BMAL1 ChIP results (Fig. 5A).

Evidence for circadian clock function in the uterus is limited but supports a contribution of the uterine clock in the process of implantation, development of the conceptus and eventual parturition (Miller et al., 2004; Dolatshad et al., 2006; Ratajczak et al., 2009; Boden et al., 2010). Johnston and colleagues were the first to demonstrate rhythmic expression of clock genes in the rodent uterus (Johnston et al., 2006a, b). Subsequent investigations determined that uterine cells were in fact semi-autonomous clocks (Nakamura et al., 2008; Ratajczak et al., 2010). The timing of clock gene expression in the rodent uterus appears to be affected by the reproductive cycle (Nakamura et al., 2010) and stimulation with ovarian steroids (He et al., 2007; Nakamura et al., 2008). In view of these data, it would be valuable to explore the potential regulation of steroid (as well as oxytocin and prostaglandin) receptors in the human uterus by clock genes. Global knockout of the core clock gene mBmal1 disrupts implantation, alters the level of steroid hormone synthesis and compromises fertility in mice (Boden et al., 2010). Furthermore, targeted deletion of mBmal1 gene expression in the mouse myometrium abrogates normal implantation (Ratajczak et al., 2010).
et al., 2012). The latter group reported that over 90% of control females but only 64% of females with disrupted myometrial mBmal1 completed parturition during the expected time window of gestation.

Likewise, human reproduction is a circadian process. The ovulatory LH surge in women generally occurs just before the start of the active (daytime) phase (Cahill et al., 1998), while labor and delivery generally occur late during the inactive (nighttime) phase, at least in part due to the circadian secretion of melatonin (Mahoney, 2010; Olcese, 2012). We have summarized this process and our current findings in a model (Fig. 6). Based on our previous studies (Sharkey et al., 2009; Olcese, 2012; Olcese and Beesley, 2014), our working hypothesis is that the expression of the MTNR1B receptor is under ambivalent transcriptional control, both positive and negative. The positive control is exerted by transcription factors, including NFAT (Olcese and Beesley, 2014) and CLOCK/BMAL1 (this study), while the negative control factors are likely to include PER/CRY and other yet-to-be identified transcriptional inhibitors. Given that myometrial MTNR1B expression is minimal until labor (Sharkey et al., 2009), it is hypothesized that during most of pregnancy, the inhibitory controls predominate. How MTNR1B expression is then released from such inhibition at the time of labor is not yet clear. However disruption of circadian rhythms (e.g. shift work or jetlag) is associated with more frequent, irregular and prolonged menstrual cycles as well as alterations in serum gonadotrophin levels, increased risk of preterm birth and reduced fecundity (Zhu et al., 2004; Miller and Takahashi, 2013). Recently, gene polymorphisms in BMAL1 and the CLOCK paralog NPAS2 have been associated with a higher risk of miscarriage (Kovanen et al., 2010). Our results provide new insights into a cellular target for clock genes in the human myometrium. Further analysis of the circadian regulation of the melatonin receptor and other signaling mechanisms in the myometrium can be expected to contribute important clues regarding the activation of term and preterm labor.

Acknowledgements

The authors thank Drs R. Ann Word and P. W. Keller for the primary myometrial cells from the Human Biological Fluids and Tissue Repository at the University of Texas Southwestern Medical Center (NIH HD11149); Dr D. Weaver for the CLOCK expression construct; Dr C. Lee for the BMAL1 antibody; Jodi Slade for her artistic depiction of our work and Dr R. Nowakowski for his continuing support.

Authors’ roles

S.B. and J.O. were involved in study conception and design. S.B., J.O. and J.L. performed the experiments. S.B. and J.O. analyzed and discussed the results, and prepared the manuscript. S.B. and J.O. revised the manuscript.

Funding

This work was supported by grants to J.O. from the Florida State University Council on Faculty Research & Creativity, and the FSU College of Medicine (Division of Research).

Conflict of interest

None declared.

Figure 6 The regulation of uterine contractions mediated through the circadian clock and hMNTR1B. hMNTR1B expression is under circadian regulation in the myometrium, regulated via the dimerization of CLOCK and BMAL1 that act on its proximal promoter to stimulate transcription. The subsequent receptor protein would be inserted into the plasma membrane, where it can be bound and activated by its natural ligand, melatonin, itself under circadian control by the brain. This interaction would trigger a cascade of intracellular signaling, in synergy with other uterotonins, which results in enhanced contraction of the uterus, leading to nocturnal parturition.
Circadian regulation of MTNR1B in myometrial cells