LACTATION BIOLOGY SYMPOSIUM: Circadian clocks as mediators of the homeorhetic response to lactation

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ABSTRACT: The transition from pregnancy to lactation is the most stressful period in the life of a cow. During this transition, homeorhetic adaptations are coordinated across almost every organ and are marked by changes in hormones and metabolism to accommodate the increased energetic demands of lactation. Recent data from our laboratory showed that changes in circadian clocks occur in multiple tissues during the transition period in rats and indicate that the circadian system coordinates changes in the physiology of the dam needed to support lactation. Circadian rhythms coordinate the timing of physiological processes and synchronize these processes with the environment of the animal. Circadian rhythms are generated by molecular circadian clocks located in the hypothalamus (the master clock) and peripherally in every organ of the body. The master clock receives environmental and physiological cues and, in turn, synchronizes internal physiology by coordinating endocrine rhythms and metabolism through peripheral clocks. The effect of the circadian clock on lactation may be inferred by the photoperiod effect on milk production, which is accompanied by coordinated changes in the endocrine system and metabolic capacity of the dam to respond to changes in day length. We have shown that bovine mammary epithelial cells possess a functional clock that can be synchronized by external stimuli, and the expression of the aryl hydrocarbon receptor nuclear translocator-like gene, a positive limb of the core clock, is responsive to prolactin in bovine mammary explants. Others showed that 7% of genes expressed in breasts of lactating women had circadian patterns of expression, and we report that the diurnal variation of composition of bovine milk is associated with changes in expression of mammary core clock genes. Together these studies indicate that the circadian system coordinates the metabolic and hormonal changes needed to initiate and sustain lactation, and we believe that the capacity of the dam to produce milk and cope with metabolic stresses in early lactation is related to her ability to set circadian rhythms during the transition period.

Key words: circadian clock, homeorhesis, lactation, photoperiod

INTRODUCTION

Approximately one-third of dairy cows experience some form of metabolic or infectious disease during the transition period (LeBlanc, 2010). From the time of dry off to the time of re-initiation of lactation, a cow often experiences multiple ration shifts, and she may also be subjected to location and social group moves. Rapid changes in both hormonal and metabolic systems also occur during the transition period. All of these changes tend to increase the level of stress, which, in turn, further affects physiology, metabolism, and immune system (for review, see Drackley, 1999; Mulligan and Doherty, 2008). Understanding how metabolic and physiological set points are established in response to changes in physiological state, nutritional status, environment, or all 3 will enable the development of simple approaches that maximize productive efficiency and minimize metabolic disturbances in dairy cows. In this paper, we review scientific literature on the function of the circadian system in coordinating internal processes and synchronizing these processes to the environment of the animal. We present our hypothesis that circadian clocks are involved in homeorhetic regulation of lactation by mediating coordinated changes in hormones and metabolism across multiple tissues in response to changes in the physiology and environment of the dam.

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HOMEOSTATIC AND HOMEORHETIC REGULATION

The overall goal of physiological regulation is to maintain the well-being of an animal regardless of life-cycle state or environmental challenges. To meet this goal, 2 conceptually distinct, but interacting types of regulation, homeostasis and homeorhesis, are involved (D. E. Bauman, Cornell University, Ithaca, NY, personal communication). Homeostasis is the natural tendency of a living organism to maintain a stable equilibrium regardless of interactions with the external environment (Ray and Phoha, 2005). In contrast, homeorhesis is the natural tendency of a living organism to continue its evolving development, albeit being possibly different under different environmental conditions (Ray and Phoha, 2005).

Homeostasis is short-term regulation representing mechanisms that enable organisms to function under a range of environmental conditions (Sauvant and Lovatto, 2000). Homeostatic regulation acts through feedback loops and is initiated by signals (e.g., change in blood glucose concentration) that trigger a cascade of metabolic processes which return the organism back to equilibrium (Sauvant and Lovatto, 2000). Homeorhesis is long-term regulation that expresses the genetic potential of the animal within a given environment (Sauvant and Lovatto, 2000). The key features of homeorhetic regulation are its 1) chronic nature (i.e., hours or days vs. seconds or minutes required for homeostatic regulation); 2) simultaneous influence on multiple tissues, and 3) mediation through altered responses to homeostatic signals (Bell et al., 1987). Bauman and Currie (1980) were the first to apply the concept of homeorhesis to describe the regulation of metabolic changes in the dam during the transition from pregnancy to lactation, and defined homeorhesis as “the orchestrated or coordinat-ed changes in metabolism of body tissues necessary to support a [dominant] physiological state.”

HOMEORHETIC ADAPTATIONS TO PREGNANCY AND LACTATION

Within the first few days after calving, the requirement for glucose, AA, and fatty acids of the mammary gland are approximately 2.2, 2.0, and 4.5 times, respectively, those of the uterus in late pregnancy (Bell, 1995). This dramatic increase in nutrient demand to support milk production in dairy cows is not supported by increased voluntary feed intake alone (Bell, 1995). During the periparturient period, homeorhetic adaptations in metabolism are coordinated across almost every organ of the cow to accommodate the increased energetic demands of lactation (for review, see Bauman and Currie, 1980; Bauman et al., 1982, 1983, 1989). Changes in the hormonal milieu of the cow mediate metabolic changes that occur during the transition period (Bell and Bauman, 1997). For example, increases in prolactin, GH, estradiol, and cortisol during the periparturient period decrease peripheral tissue insulin sensitivity and responsiveness. These changes in insulin homeostasis result in increased rates of liver gluconeogenesis and decreased rates of glucose uptake by adipose and muscle, decreased adipose lipogenesis, and decreased protein synthesis in muscle with concomitant increases in protein degradation and AA release (Bell and Bauman, 1997). Although these and other homeorhetic adaptations are well characterized in the dairy cow, we do not know how they are coordinated and synchronized across multiple systems.

Similar to ruminants, rodent dams undergo homeorhetic adaptations to accommodate the increased nutrient and energetic demands of lactation (Williamson, 1980, 1986; Augustine et al., 2008). We used rats as models to study homeorhetic regulation during the periparturient period and collected mammary, liver, and adipose tissues from dams on d 20 and 21 of pregnancy, during prelabor, labor, and at birth, and on d 1 and 3 of lactation (for details, see Plaut et al., 1999; Ronca et al., 2003; Lintault et al., 2007; Casey et al., 2009; Patel et al., 2011). Lipid synthetic capacity for all 3 tissues was determined by measuring the rate of incorporation of uniformly labeled 14C-glucose into lipids. Within an 8-h period encompassing prelabor, labor, parturition, and initiation of suckling, the rate of lipid synthesis increased 6-fold in the mammary gland (Figure 1). Lintault et al. (2007) showed changes in lipid synthetic rates also occurred in liver and adipose during the transition from pregnancy to lactation. Plasma samples taken from these same dams showed dynamic swings in concentrations of blood triglycerides. These changes were accompanied by changes in dam feed intake, BW, and ME (Lintault et al., 2007).

Global transcriptional analysis was used to investigate how gene expression changed across multiple tissues during the transition from pregnancy to lactation (Casey et al., 2009; Patel et al., 2011). Total RNA was isolated from mammary, liver, and adipose tissues from rat dams on pregnancy d 20 and lactation d 1, and Affymetrix gene chips were used to measure gene expression. We found that genes changing expression from late pregnancy to the onset of lactation enriched multiple pathways and gene ontologies related to energy homeostasis as well as gene sets classically associated with central nervous system reception and integration (Casey et al., 2009; Patel et al., 2011).

To gain insight into what was stimulating these changes, we took a closer look at genes commonly up-regulated at the onset of lactation clustered in the gene ontology, transcription regulator activity. One hundred twelve genes commonly upregulated among mammary, liver, and adipose during the transition from pregnancy to lactation clustered in transcription regulator activity and included 2 core circadian clock genes, aryl hydrocarbon receptor nuclear translocator-like (ARNTL, also known as BMAL1) and circadian locomotor output cycles kaput (CLOCK). When we examined genes commonly downregulated in mammary, liver, and adi-
pose tissues during the transition from pregnancy to lactation, we found that the gene ontology, rhythmic process, was significantly enriched with downregulated genes including \textit{NR1D1} (nuclear receptor subfamily 1, group D, member 1 or Rev-erbα), albumin D-box binding protein (\textit{DBP}), and \textit{BHLHB2} (basic helix-loop-helix domain containing, class B 2). All 3 are well-characterized transcriptional targets of the core circadian clock gene products \textit{CLOCK} and \textit{ARNTL}. Based on these signatures, we decided to look more closely at the circadian system as a possible regulator of homeorhetic changes that occur during the transition period.

\textbf{WHY CIRCADIAN CLOCKS?}

Living organisms evolved a circadian system, internal biological clocks that generate circadian rhythms, to help their bodies adapt to the daily cycle of day and night (i.e., light and dark), which result from the rotation of the earth every 24 h. Circadian rhythms are roughly 24-h cycles in physiology and behavior, and include cycles in body temperature, sleep-wake patterns, hormonal secretion, and daily activity (Hastings et al., 2007). Figure 2 illustrates characteristics of circadian rhythms. The period of a rhythm is approximately 24 h in mammals and is the length of time from peak to peak (or trough to trough). The phase of a circadian rhythm reflects where the peak and the trough occur (e.g., the peak and trough of performance within the 24-h period). The amplitude is the difference from the peak and trough. Both external and internal cues can cause changes in metabolic and physiological rhythms (Figure 2). Changes include phase shifts, which can result from changes in light exposure; change in amplitude, which may reflect the relative strength of the underlying pacemaker; and a change in period (Bass and Takahashi, 2010).

The function of the circadian system is to coordinate internal physiological processes and synchronize these processes with the environment of the organism to ensure that physiological processes are performed at the appropriate and optimal time of day or night (Froy, 2010). The central circadian clock is located in the suprachiasmatic nuclei (SCN) of the hypothalamus in the brain. The SCN clock is composed of multiple, single-cell circadian oscillators, which generate coordinated circadian outputs that regulate overt rhythms in physiology and behavior (Froy, 2010). Rhythmic oscillations generated by the SCN are not exactly 24 h and therefore require regularly occurring environmental signals or Zeitgebers to prevent drifting (or free-running) out of phase (Froy, 2010).

\textbf{Figure 1.} Glucose incorporation into lipids in the mammary gland increased 6-fold from prelabor to initiation of suckling, a period of time that is less than 8 h. Abdominal number 4 mammary glands were removed from anesthetized dams at each stage: prelabor, labor, parturition, and initiation of suckling (n = 6/stage). Glucose incorporation into lipids was calculated and expressed as nanomoles of glucose used per 100 mg of tissue per 1 h of incubation.

\textbf{Figure 2.} Characteristics of circadian rhythms and the manner in which rhythms can be disrupted. A) The period of a rhythm is approximately 24 h in mammals and is the length of time from peak to peak (trough to trough); the phase of a circadian rhythm reflects where the peak and the trough occur, for example, the peak and trough of performance within the 24 h; and the amplitude is the difference from the peak and trough. The green (gray) circadian rhythms relative to blue (black) demonstrate B) a shift in the phase, changes in light exposure are known to cause shifts; C) a change in amplitude, which may reflect the relative strength of the underlying pacemaker; and D) a change in period, in particular a lengthening of the period is associated with excess fatness. The white and black alternating bar below rhythms indicates light and dark phases of a 12-h light, 12-h dark cycle. Color version available in the online PDF.
Homeorhesis and circadian clocks

The light-dark (LD) cycle is the most important environmental cue for entraining the central circadian clock (Reppert and Weaver, 2002). Light is perceived by the retina, and the signal is transmitted via the retinohypothalamic tract to the SCN (Reppert and Weaver, 2002). The central clock coordinates peripheral clocks located in every organ of the body. The SCN sends signals to peripheral oscillators to coordinate and synchronize rhythms across the organism via neuronal connections or circulating humoral factors (Froy, 2010).

Complete destruction of SCN neurons abolishes overall circadian rhythmicity in peripheral tissues. The loss of rhythmicity is thought to be due to loss of synchrony among individual cells in peripheral tissues because at the individual cell level each cell oscillates, but with a different phase. Thus, the central circadian clock is often termed the master clock that synchronizes all peripheral clocks found within non-SCN cells of the organism, including other regions of the central nervous system (Froy, 2010).

THE WORKINGS OF BIOLOGICAL CLOCKS

Biological clocks can be viewed as having 3 parts: 1) a way to receive input from the environment to set the clock (e.g., firing of the retinal tract upon light stimulation or binding of hormone to cell surface receptor); 2) the clock itself; and 3) genes that help the clock control output. The clock itself is composed of an auto-regulatory transcriptional-translational molecular feedback loop of positive and negative factors that results in oscillating expression of core clock genes. In mammals, the core clock genes are conserved (for review, see Reppert and Weaver, 2002). In the primary feedback loop, CLOCK and ARNTL or NPAS2 and ARNTL heterodimerize in the cytoplasm to form a complex that, after translocation to the nucleus, initiates transcription of target genes by binding to the E-box element (Figure 3). The CLOCK:ARNTL or NPAS2:ARNTL heterodimers drive rhythmic expression of numerous genes including their own repressors, the core clock period genes (PER1, PER2, and PER3) and cryptochrome genes (CRY1 and CRY2). Negative feedback is achieved by PER:CRY heterodimers that translocate back to the nucleus to repress their own transcription by inhibiting the activity of the CLOCK:ARNTL (NPAS2:ARNTL) complexes. The expression of ARNTL is also regulated by 2 of its transcriptional targets, nuclear receptors Rev-erba (NR1D1) and Rora (RORA/NR1F1), which respectively repress or activate ARNTL transcription by competing for their specific response elements [RORE (Guillaumond et al., 2005)]. In recent years, new information about posttranscriptional regulation in the circadian system has been discovered. Such regulation has been shown to alter the phase and amplitude of rhythmic mRNA and protein expression in many organisms including mammals (for review, see Koijima et al., 2011).

AFFECTENTS AND EFFERENTS OF THE SCN

The SCN functions as a key integrator of both environmental and internal signals (Kuhlman and McMahon, 2006). In turn the SCN temporally organizes a diverse range of physiological processes, including metabolism, growth, and cortical arousal, and in this way enables organisms to optimize performance during waking and tissue repair and memory consolidation during sleep. The SCN achieves this regulation via input and output pathways. Information sent to and from the SCN affect and mediate homeostasis and homeostatic set points (Nakagawa and Okumura, 2010), indicating that the circadian system plays a key role in mediating homeorhetic responses to changes in both physiology and the environment.

Proper entrainment of the central clock requires input signals that include light, food intake, and activity. The entraining signals from light, nutrients, and locomotor activity are relayed to the SCN via direct projections from the retina and arcuate nucleus and raphe nucleus of the hypothalamus, respectively (for details, see Figure 4). The 2 major ways metabolic information reach the SCN are 1) the sympathetic and parasympathetic branches of the autonomic nervous system; and 2) hormones or nutrients, such as glucose, that cross the blood-brain barrier (Froy, 2010).

Figure 3. Transcriptional and translational autoregulatory feedback loops that regulate circadian rhythms in cells. The positive loop consists of aryl hydrocarbon receptor nuclear translocator-like (ARNTL) and circadian locomotor output cycles kaput (CLOCK) gene products or ARNTL and NPAS2 gene products and the negative loop consists of the Per and Cry gene products. The CLOCK and ARNTL proteins heterodimerize to bind to the E-box to activate transcription of numerous target genes, including their own repressors, PER and CRY, thus forming a transcriptional feedback loop. The expression of ARNTL is also regulated by 2 of its transcriptional targets, nuclear receptors Rev-erba and Rora, which respectively repress or activate ARNTL transcription by competing for the same promoter element (RORE) and forming a secondary interlocked feedback loop.
mediate outflow of SCN information is primarily limited to the medial hypothalamus, and here the SCN signal is translated into hormonal and autonomic signals for peripheral organs (for details, see Figure 4; Kalsbeek et al., 2010, 2011). Neural connections from the SCN control peripheral rhythms in hormone release and energy metabolism (Kalsbeek et al., 2010). For example, outputs from the SCN to the PVN result in the circadian patterns of corticotropin-releasing hormone secretion. Corticotropin-releasing hormone in turn stimulates ACTH release from the anterior pituitary gland, which stimulates synthesis of cortisol in the adrenal gland. Circadian oscillation of plasma cortisol communicates time of day to peripheral tissues.

Neurons emanating from the SCN also stimulate neurons that project to the spinal column, which in turn stimulate preganglion sympathetic neurons that innervate the pineal gland. In response to this relayed signal initiated by the SCN, melatonin is synthesized in the pineal gland according to the length of the photoperiod. Melatonin in turn functions as a biochemical transducer of photoperiodic information to all cells in the body (including SCN neurons), and changes in duration and amplitude of nocturnal melatonin secretion serve to signal seasonal variations of day/night cycle length (for review, see Simonneau and Ribelayga, 2003). Circadian oscillation of core body temperature, which is regulated by the SCN, is believed to also serve as an output signal that influences the timing of peripheral clocks (Buhr et al., 2010). With this in mind, it is intriguing to speculate that the circadian system mediates homeorhetic changes needed for adaption to heat stress (Collier et al., 2006).

**PERIPHERAL CLOCKS**

Molecular clocks are expressed in every tissue of the body including other areas of the brain (Hastings et al.,
The specific input signals and outputs of these core molecular clocks are less well defined than those of the SCN. Inputs to peripheral clocks likely include humoral and neuronal information sent from SCN as well as timing of food intake. Global temporal expression profiles of liver, adipose, mammary, and heart tissues revealed that 3 to 10% of genes expressed in these tissues exhibited circadian patterns and were found to be involved in rate-limiting steps critical for organ function (Akhtar et al., 2002; Panda, 2002; Storch et al., 2002; Ando et al., 2003; Maningat et al., 2009; Sookoian et al., 2010) indicating that peripheral clocks are important in regulating organ output.

The liver clock has been the best characterized of all peripheral clocks. Genes with coordinated circadian expression in the liver were found to encode molecules involved in metabolism of sugar, lipid, and cholesterol (Akhtar et al., 2002; Panda, 2002; Storch et al., 2002). In the rodent liver, 35% of clock-regulated genes direct biosynthesis and metabolism and 10% regulate gene transcription (Akhtar et al., 2002). These include transcription factors and nuclear hormone receptors that regulate glucose and lipid homeostasis (Brewer et al., 2005; Yang et al., 2006). Peripheral liver clocks have been shown to be regulated by melatonin, glucocorticoids, glucagon, and insulin (Stokkan et al., 2001; Ruiter et al., 2003; Kennaway et al., 2006; Kohsaka and Bass, 2007). Thus, hormonal pattern coordinates liver metabolism with time of day and nutritional status of the animal. Rodents with clock gene mutations have dysfunctional glucose homeostasis, insulin secretion and sensitivity, and fat and cholesterol metabolism (Rudic et al., 2004; Turek et al., 2005). Similarly, SNP in Clock and Arntl genes in humans are associated with abnormal hepatic fat and glucose metabolism (Sookoian et al., 2007; Woon et al., 2007).

FOOD ENTRAINABLE OSCILLATOR

Although calorie/energy restriction entrains the SCN clock, timed meals entrain peripheral oscillators, particularly in the liver (Froy, 2007). When food is available only for a limited time each day, rats increase their locomotor activity 2 to 4 h before the onset of food availability (Stokkan et al., 2001). This food anticipatory behavior occurs in other mammals and is accompanied by increases in body temperature, secretion of cortisol, gastrointestinal motility, and activity of digestive enzymes (Stokkan et al., 2001). Further, rats with SCN-lesions expressed circadian rhythms in body temperature and corticosterone secretion when restricted to 1 meal per day, indicating that although the SCN and circadian rhythms can be entrained to daily light-dark cycles, rhythms in animal behavior and physiology are also affected by the timing of food availability (Yamazaki et al., 2000; Stokkan et al., 2001; Stephan, 2002; Schibler et al., 2003). These findings and others led investigators to search for a food entrainable oscillator.

The dorsomedial hypothalamic nucleus (DMH), a major target of the SCN, may function as the food entrainable oscillator (Herzog and Muglia, 2006). The DMH receives both neural and humoral input from previously characterized pathways important for the regulation of feeding, BW and energy consumption. The DMH, in turn, projects to brain regions critical for the regulation of sleep and wakefulness, body temperature, and cortisol secretion (Gooley et al., 2006). Restricting rats to a single daytime meal shifted the circadian peak of neuronal activity in the DMH to time of feeding. Further, lesioning the DMH eliminated the premeal rise in body temperature and wakefulness, and severely attenuated the premeal rise in locomotion (Gooley et al., 2006). These data indicate that scheduled daytime feeding entrains circadian rhythms by altering the phase of rhythmic activity in the DMH, thereby shifting behavioral and physiologic circadian rhythms.

To systematically dissect the role that food, feeding pattern, and the circadian system have in determining rhythmic gene expression in liver, changes in temporal patterns of global transcription were measured in response to fasting and re-feeding in wild-type C57Bl6 mice and oscillator-deficient mice (cry1−/−; cry2−/−; Vollmers et al., 2009). Circadian gene expression profiles of these mice were examined under 3 different conditions: ad libitum, daytime-restricted feeding, and prolonged fasting. In oscillator-deficient Cry mutant mice, restricted feeding stimulated 24-h rhythms in gene expression of 617 transcripts, which were identified as the food-only oscillating transcripts. In wild-type mice that were fasted, 368 transcripts showed circadian patterns of expression, indicating that expression of these genes was driven by the circadian clock. When wild-type mice were restricted to daytime feeding, 4,960 transcripts displayed rhythmic expression, thus demonstrating that transcriptional synergy of hepatic rhythms occurs when access to feed is restricted without changing total caloric intake and results in more robust metabolic and circadian rhythms (Vollmers et al., 2009).

These studies indicate that there is an independent but interactive organization that links metabolic controls with the central circadian system and that proper feeding time may synchronize/coordinate compatible temporal events in metabolism with other rhythms of behavior and physiology leading to more robust rhythms (Kuhlman and McMahon, 2006). Studies on the effects of inappropriate meal-time eating in shift workers indicate that it leads to metabolic disease (Bass and Takahashi, 2010). It remains to be determined if temporally restricting feeding or coordinating feeding or both with other physiological rhythms can have beneficial effects on production efficiency in cattle. Recent work in cattle showed that circadian pattern of milk synthesis in dairy cows was responsive to the timing of feed intake (Rottman et al., 2011). These findings point to the importance of understanding the impact of feeding time on the circadian system to identify dairy management plans that optimize production and animal welfare.
SEASONAL CLOCKS

Changes in day length, or photoperiod, provide a reliable and predictive indicator of seasonal changes in environmental conditions (Dardente et al., 2010). Many mammals exhibit annual rhythms (i.e., circannual rhythms) in physiology and behavior, including reproduction, pelage (e.g., wool growth), and energy balance that are regulated by changes in hormonal milieu which are stimulated by changes in photoperiod (Dardente et al., 2010). As discussed previously, perception of photoperiodic information begins with light reception by the retina and neurotransmission to the SCN. The SCN communicates photoperiodic information to the pineal gland via a multisynaptic pathway that stimulates synthesis and release of melatonin. This neural pathway drives both circadian and annual (i.e., seasonal) rhythms of melatonin release. Light acutely decreases melatonin secretion, such that melatonin secretion occurs at night and has a greater duration during short-day photoperiods. Melatonin, in turn, activates specific receptors localized in discrete regions of the brain and pituitary gland, which regulate downstream hormone secretion (Dardente et al., 2010).

Seasonal hormonal rhythms of prolactin and thyroid hormones are generated from the pars tuberalis, the sheath around the pituitary stalk extending from the hypothalamus to the pars distalis of the anterior pituitary, where the majority of pituitary hormones are synthesized. An increased density of melatonin receptors are expressed on the pars tuberalis, and seasonal changes in melatonin concentrations have been shown to correlate with seasonal changes in the pars tuberalis transcriptome (Lincoln et al., 2006; Dardente et al., 2010). A full complement of clock genes are expressed in the ovine pars tuberalis, and undergo 24-h cyclical expression (Dardente et al., 2010). Activation of Per genes occurs in the early day, whereas activation of Cry genes occurs in the early night. This temporal association is evident under both long and short days; thus, the Per-Cry interval varies directly with photoperiod. Because PER:CRY, protein:protein interactions affect stability, nuclear entry, and gene transcription, the change in phase of Per and Cry expression provides a mechanism for decoding the long-day/short-day melatonin signal (Lincoln et al., 2006; Dardente et al., 2010).

Across many mammalian groups, long photoperiods stimulate, and short photoperiods inhibit prolactin secretion (Lincoln et al., 2006; Duncan, 2007). Photoperiod effects on prolactin secretion are regulated by changes in the 24-h melatonin profile that binds to its receptors on the pars tuberalis. The currently accepted view of photoperiodic regulation of prolactin secretion is that melatonin regulates the secretory function of the pars tuberalis, which secretes prolactin-releasing factors (i.e., tuberalins), and these paracrine factors stimulate lactotrophs to secrete prolactin (Lincoln et al., 2006; Duncan, 2007).

The regulation of seasonal rhythms of thyroid hormones through the pars tuberalis is distinct from the mechanisms that regulate photoperiod changes in prolactin secretion. Similar to prolactin, the initiation of the signal begins with seasonal transcriptional effects of melatonin on cells within the pars tuberalis. In particular, expression of β-thyroid-stimulating hormone (TSH) has been shown to be regulated in the pars tuberalis by melatonin in sheep and hamsters and to show seasonal variation with less expression during short days (Dardente et al., 2010). In turn, TSH acts by a retrograde mechanism on TSH receptor-expressing cells in the basal hypothalamus (Ono et al., 2008). The unusual direction of information flow probably reflects an ancestral mechanism preceding the evolution of a separation between the hypothalamus and pituitary gland and the development of a local portal blood system linking the tissues (Hanon et al., 2008). Studies in birds and mammals indicate that central thyroid hormone availability may not only play a significant role in the seasonal regulation of energy metabolism but also the reproductive axis (Nakao et al., 2008).

In sheep, melatonin also acts within the hypothalamus to mediate control of seasonal changes in gonadotropin secretion and gonadal activity (Lincoln and Richardson, 1998). Administration of melatonin to maintain sustained increased concentrations in anestrous ewes led to the activation of the hypothalamic-pituitary GnRH-LH axis. Activation of this gonadotrophic system in anestrous ewes, leading to the onset of estrus, requires several weeks of exposure to melatonin, during which a change in the feedback action of estradiol on GnRH and LH secretion plays a pivotal role (Kennaway, 1988). In situ hybridization and autoradiography showed that melatonin receptors are expressed on the preprimamillary hypothalamic area and showed circadian oscillations in expression (Migaud et al., 2005).

Photoperiod changes in hormonal milieu in turn affect peripheral tissues. For example, a transition from short-day to long-day photoperiod in sheep resulted in changes in rhythm amplitude of mRNA abundance of the core clock gene ARNTL and shift in the phase of PER2 expression in the liver (Andersson et al., 2005). The shifting pattern of PER2 mRNA correlated with daily rhythms of plasma cortisol, indicating that cortisol regulates timing of PER2 expression in the ovine liver (Andersson et al., 2005). Photoperiodic treatment of cattle altered liver mRNA abundance of multiple metabolic enzymes known to show circadian patterns, including acetyl-CoA carboxylase, phosphoenolpyruvate carboxykinase, and fatty acid synthase (Auchtung et al., 2003; Connor et al., 2007).

Although dairy cows are not seasonal breeders, photoperiod has been shown to affect growth and milk production in cattle (Dahl et al., 2000, 2002; Collier et al., 2006). Dairy cows exposed to long-day photo-
period increased milk yield (Peters et al., 1978), and exposure to short-day photoperiod during the dry period enhanced subsequent lactation performance (Dahl, 2008). These photoperiod effects on lactation are due in part to changes in mammary cell proliferation, as well as immune and metabolic capacity of the animal, and are believed to be mediated in part by seasonal changes in prolactin and prolactin signaling (Auchtung et al., 2003, 2005; Dahl, 2008).

Studies focused on understanding the endocrine effects of altering photoperiod in cattle have shown that circulating concentrations of prolactin increased and melatonin decreased when heifers and dairy cows were exposed to long-day photoperiod (Peters et al., 1978, 1981). Exposure to short-day photoperiod during the dry period depressed prolactin secretion but increased prolactin receptor mRNA abundance in mammary, liver, and immune tissues (Dahl, 2008). However, when prolactin was administered exogenously to lactating dairy cows there was no effect on milk production (Plaut et al., 1987). The lack of effect of prolactin in this study may have been due to the timing of prolactin administration relative to the circadian clocks or season or both. When slow release melatonin implants were administered for 12 wk during long-day photoperiods, plasma concentrations of prolactin were decreased and accompanied by a 23% reduction in milk yield (Auldist et al., 2007). However, the regulatory relationship of melatonin with seasonal prolactin is not as clear cut in cattle as in other species. Pinealectomy or melatonin feeding or infusion did not affect prolactin plasma concentrations in bull calves (Stanisiewski et al., 1988a,b). In contrast, melatonin fed to prepubertal heifers in the middle of long-day photoperiod decreased mean serum prolactin by 27% and decreased DNA content and concentration in mammary parenchyma (Sanchez-Barcelo et al., 1991). When melatonin was fed to heifers to mimic a short days, IGF-I induction by long days was suppressed but there was no effect on milk yield in cows (Dahl et al., 2000).

Annual rhythms of energy balance and reproduction evolved so that seasonal changes in the external environment can be anticipated to enable animals to adjust their physiology and behavior in preparation for the changing demands of the environment (Ebling and Barrett, 2008). These photoperiod studies and others indicate neuroendocrine functions that regulate reproduction and energy balance are tightly integrated with each other and biological clocks (Silveyra et al., 2010). Seasonally coordinated changes in circulating concentrations of hormones and metabolic capacity (e.g., hepatic enzyme activity and milk production) indicate that the photoperiod effect on animal growth and production is mediated by homeorhetic regulators (D. E. Bauman, Cornell University, Ithaca, NY, personal communication). A greater understanding of environmental influences on the inner biological clock may provide practical ways to alter production and health in cows as they transition into lactation (Dahl, 2008).

**THE CIRCADIAN SYSTEM IN REPRODUCTION AND LACTATION**

In the study introduced previously, we found that transcriptomes of all 3 tissues showed changes in molecular clock genes during the transition from pregnancy to lactation. Specifically, there was an induction of the positive limb core clock and clock regulatory genes (i.e., ARNTL, NPAS2, CLOCK, and RORA) and a suppression in the negative limb of clock-related genes (i.e., BHLHB2, NR1D1, CSNK1E) in mammary, liver, and adipose tissue, revealing coordinated changes in molecular clocks among multiple tissues during the transition from pregnancy to lactation (Casey et al., 2009). However, our gene expression data were from a single time point in each physiological state (i.e., approximately 6 h after the light phase began on d 20 of pregnancy and d 1 of lactation); thus, changes in molecular clocks may have been due to an amplitude change or shifts in circadian pattern of gene expression. A study done by Metz et al. (2006) used circadian sampling (i.e., tissues were collected every 6 h over a 24-h period) and showed that there was an increase in amplitude of mRNA abundance of the core clock genes ARNTL and CRY1 in mammary tissue from lactating mice relative to 10-d-old virgin mice. These findings indicated to us that as the mammary gland develops and differentiates, there is an increase in amplitude of core molecular clock gene circadian expression. This is an important finding because the amplitude of a rhythm is believed to be indicative of the relative strength of the circadian clock (Vollmers et al., 2009).

Work of others further confirmed the presence of a mammary circadian clock. Global temporal gene expression analysis of RNA isolated from human milk fat globules revealed that 7% of the genes expressed in the lactating breast of women showed circadian patterns of expression (Maningat et al., 2009, 2011). The core clock genes, PER1, PER2, PER3, CRY1, CRY2, CLOCK, ARNTL, and CSNK1E, were among the genes that changed expression over time. The top molecular functions associated with genes that showed circadian patterns of expression were cell development, growth and proliferation, morphology, assembly and organization, and cell movement. Two canonical pathways significantly enriched with genes showing circadian patterns of expression in lactating glands were chondroitin sulfate biosynthesis and fatty acid elongation in mitochondria (Maningat et al., 2009).

Preliminary in vitro studies in our laboratory using bovine mammary cells showed that external stimulation synchronized mammary clocks, and expression of the core clock gene, ARNTL, was induced by lactogens. Using the approach of Maningat et al. (2009) we have begun to characterize the circadian pattern of gene expression in lactating dairy cows by isolating RNA from the milk fat globule. Our preliminary data revealed that similar to humans, the core clock genes PER2 and ARNTL showed circadian patterns of
expression in dairy cows. Circadian patterns of milk urea nitrogen and percent fat were also evident (data not shown). These results indicate that mammary clock genes and milk components exhibit circadian rhythms and support further the suggestion that changes in gene expression coincide with the diurnal changes in milk composition. Interestingly, one of the most striking features of female clock/clock mutant mice, which have disrupted circadian rhythms, appears to be an inability to adequately nourish their young (Dolatshad et al., 2006). Our studies with clock/clock mutant mice have found that this may be due in part to poorer mammary development (our unpublished data). Based on these findings, we believe that the circadian clock in the mammary gland functions to control a set of genes important to mammary function.

The circadian system plays a key role in the timing of reproductive events (for review, see Sellix and Menaker, 2011). Lesions of SCN result in infertility in rodents because of the lack of the ability to synchronize events for ovulation (Turek et al., 1984), and mice with mutant core clock genes or core clock gene knockout mice exhibit reduced fertility and fecundity (Dolatshad et al., 2006; Pilorz and Steinlechner, 2008; Ratajczak et al., 2009; Boden et al., 2010). Because lactation represents the continuum of reproduction in mammals, it is possible the circadian system plays a role in timing the coordinated changes in hormonal milieu and metabolism needed to initiate lactation. Our finding that changes in molecular clocks occur in multiple tissues during the transition from pregnancy to lactation supports this hypothesis (Casey et al., 2009; Patel et al., 2011), as well as the fact that clock/clock mutant mice cannot adequately nourish neonates during lactation (Dolatshad et al., 2006). Further, because core molecular clocks regulate important rate-limiting processes important to the function of organs, changes in peripheral clocks during the transition from pregnancy to lactation are likely to play a key role in changing the metabolome of the dam during the periparturient period to support milk synthesis.

SUMMARY AND CONCLUSIONS

Homeorhetic controls are based on time-dependent changes in metabolism to establish a new physiological state, such as pregnancy or lactation. Likewise the ability of cows to adapt to changes in their environment including photoperiod, heat, stress, and nutrition also require time-dependent integrative homeorhetic regulation (D. E. Bauman, Cornell University, Ithaca, NY, personal communication). We envision that during the transition period, the circadian system is modified by environmental and physiological cues that it receives. In turn, the central clock in the SCN coordinates changes in endocrine milieu and sends signals to peripheral tissues. These signals stimulate changes in core clock genes in peripheral tissues including mammary, liver, and adipose tissues, which, in turn, effectively change the proteome and metabolome of the dam to support lactation. Further, it is likely that the circadian system coordinates the metabolic and hormonal changes needed to initiate and sustain lactation, and the capacity of the dam to produce milk and cope with metabolic stress during lactation is related to her ability to set circadian rhythms.

Studies designed to determine the biochemical and molecular mechanisms that mediate homeorhetic changes in dairy cattle may enable development of new strategies to enhance efficiency of milk production and promote animal health and well-being. Research focused on examining the impact of circadian clocks as a fundamental homeorhetic mechanism that resets homeostasis in response to environmental changes may prove to maximize animal performance and minimize animal health-related issues that occur, particularly in early lactation. Disruption of circadian rhythms is known to cause metabolic disease in humans and rodent models. The responses of lactating ruminants to disruption of the circadian system are likely to be unique from rodents and humans, and findings may affect management practices on dairy farms.

LITERATURE CITED


