Melatonin is a neurohormone synthesized and released from the pineal gland with both a daily and an annual rhythm controlled by the biological clock. The dual rhythm of melatonin is robust, reproducible, and, apart from light exposure at night, hardly altered. It is a strong and reliable clock output involved in the synchronization of circadian and seasonal functions. The physiological role of melatonin in the synchronization of seasonal function is long established, and recent studies are now deciphering the molecular and cellular mechanisms involved. However, the role of melatonin in the synchronization of daily functions is less clear and still in dispute because the doses required to observe significant effects are above physiological concentrations. Additionally, a few studies suggest that melatonin may be used by the mother to tell time to their fetus. In their paper published in this issue of Endocrinology, Torres-Farfan et al. (1) show that the clock in the fetal adrenal is indeed synchronized by melatonin pulses.

**Melatonin is a robust endocrine clock output**

A number of biological functions display daily and seasonal variations in a way to anticipate and adapt to the upcoming cyclic changes in environmental conditions (light, temperature, food availability). The activity of a circadian biological clock, located in the suprachiasmatic nuclei of the hypothalamus (SCN; a small paired nucleus sat at the top of the optic chiasma) is central to these adaptive processes. The SCN clock is a strong autonomous oscillator cycling with a period of about 24 h generated by interlocked loops of clock genes coding for various transcription factors: CLOCK/BMAL1 (circadian locomotor output cycles kaput/brain and muscle ARNT-like protein 1) being the positive loop, PER/CYP (period/cryptochrome) being the negative loop, and both being modulated by other inhibitory (RORα, retinoic acid receptor-related orphan receptor) or stimulatory (REV-ERBα, reverse viral erythroblastosis oncogene product alpha) transcription factors (2). Although various experiments have formally demonstrated the pivotal role of the SCN in driving biological rhythms, virtually all other organs/structures of an organism display a similar clockwork, and this is particularly true for endocrine structures like the pineal gland, the pituitary, and the adrenal gland (3). The non-SCN clocks, however, are less robust and require resynchronization to cycle properly. The role of these secondary clocks/oscillators is still not well defined, but the current hypothesis is that they are part of a multisioscillatory system that allows temporal homeostasis of the whole organism.

To tell the body the real time, the SCN clock first needs synchronizing with the cycling environment and second to forward this information to the whole organism. Daily and seasonal variations in light intensity are the most potent synchronizing signals allowing the clock to cycle with a 24-h period exactly aligned with seasonal alteration. Notwithstanding, other cues like food intake, stress, and social events are known to impact on clock oscillations and participate in the synchronization process (4). The timing information built into the SCN uses various nervous and endocrine pathways to dictate time to the rest of the body (5). Of interest in the present context is the autonomous nervous pathway going through the hypothalamic paraventricular nuclei, the intermediolateral cells of the spinal chord, and the superior cervical ganglia to send noradrenergic fibers to the pineal gland (Fig. 1). Noradrenalin is released in the pineal gland only during the...
Melatonin synchronizes reproduction with the seasons

Anticipating the upcoming seasons to adapt physiological and behavioral functions is crucial to allow individual survival and species perpetuity. This is especially true when considering reproductive activity because it is critical that, irrespective of the duration of gestation, the offspring are born in early spring. Since the 1960s, various experimental paradigms using melatonin timed infusions and/or pinealectomy have formally demonstrated that in mammals, the photoperiodic/annual variation in circulating levels of melatonin synchronize reproductive activity with the seasons (8, 9). For many years, a few structures have been proposed to be the site of melatonin action for the synchronization of reproductive activity, particularly the pars tuberalis and some nuclei in the mediobasal hypothalamus (Fig. 1).

Recent studies have highlighted the pivotal role of the pars tuberalis in mediating the photoperiodic melatonin signal to reproductive centers in the brain. Pars tuberalis cells display a high density of melatonin receptors and are highly sensitive to melatonin. Expression of the Cry1 gene is strongly increased, and all other clock genes are inhibited, by melatonin, leading to a different pattern of clock gene expression under long and short photoperiods (10). Importantly, melatonin regulates other transcription factors governing the synthesis and release of the thyroid stimulating hormone (TSH) at levels that are increased under long photoperiod and reduced under short photoperiod (11–13). Pars tuberalis-derived TSH, by a retrograde mechanism, acts on TSH receptors located in a specific subset of ependymal cells lining the basal part of the third ventricle, known as tanycytes, to regulate deiodinase 2/3 activity, which in turn promotes the local conversion of T4 into bioactive T3 (14–16). In Siberian hamsters, hypothalamic T3 implants prevent the short photoperiod-induced inhibition of reproductive activity (17). The mechanisms by which T3 regulates reproductive activity are as yet unknown. Besides this local TSH/T3 system, we have shown that hypothalamic neuropeptides, kisspeptins in the arcuate nucleus and RF (arginine phenylalanine)-amide related peptides in the dorsomedial hypothalamus, are strongly down-regulated by melatonin in short photoperiod in the Syrian hamster (18–20). This is most interesting because both peptides are known to act upstream of the GnRH neurons to regulate reproductive activity. Kisspeptin is a very potent stimulator of GnRH release, and we have shown that central chronic infusion of this peptide is able to reverse the photo-inhibited reproductive activity of Syrian hamsters (18). Whether melatonin is acting directly on kisspeptin- and/or RF-amide related peptide-expressing neurons, or via the pars tuberalis-controlled TSH/T3 message, remains to be determined.
Melatonin displays chronobiotic properties

The marked daily variation in circulating melatonin levels has led to the hypothesis that melatonin may also drive/synchronize daily rhythms (21, 22). Indeed, peripheral administration of exogenous melatonin is able to synchronize the free-running locomotor activity of rats kept in constant darkness (23) and to induce a phase advance of endogenous melatonin secretion (24). However, these synchronizing effects of melatonin always require doses 100- to 1000-fold higher than the physiological nighttime levels. In addition, melatonin is effective only when given at the light/dark transition (onset of locomotor activity in rats), that is, when there is no secretion of endogenous melatonin. Nevertheless, this chronobiotic property of melatonin is used in humans, for example, in blind people to help them synchronize their free-running sleep-wake activity with the day/night cycle (25).

Although any central or peripheral structure expressing melatonin receptors may be involved, a number of studies point to the SCN as the main target for this chronobiotic effect of melatonin (Fig. 1). First, the SCN express high-affinity melatonin receptors in most species (6); second, the in vitro circadian rhythm of electrical activity recorded in SCN-containing hypothalamic slices is phase shifted by a bolus of melatonin given at the end of the subjective day (26); third, melatonin does not entrain the free-running locomotor activity of species with no or low density of melatonin receptors in the SCN (27); fourth, in vivo melatonin infusion at the level of the SCN by reverse microdialysis displays the same effect as peripheral administration (24). However, unlike light and other nonphotic synchronizing inputs, physiological or pharmacological doses of melatonin show no effect on clock gene expression in the SCN (10, 28). Melatonin may thus affect post-translational mechanisms or act on a clock-regulated gene in the SCN. Although the chronobiotic property of high doses of melatonin has been clearly demonstrated, the physiological role of the daily variation in circulating melatonin remains to be established, at least in adults.

Melatonin synchronizes fetal oscillators

Melatonin readily crosses the placental barrier without alteration, and melatonin binding sites have been observed in central and peripheral fetal tissues (6). In rodents, melatonin binding sites are observed in the fetal pituitary by gestational d 15 and in the SCN by gestational d 18. Therefore, it has been hypothesized that maternally derived melatonin could be used to give daily time cues to the fetus (29). Furthermore, in the new circadian scheme of the body being a multisioscillatory system, maternal melatonin could also be used to synchronize circadian oscillators of each fetus within a same litter with environmental light. Indeed, absence of maternal melatonin at early stages of gestation disrupts rat pup’s drinking behavior, and this effect is reversed by exogenous melatonin treatment to the mother (30).

In the study published in this issue of *Endocrinology*, Torres-Farfan et al. (1) have explored whether melatonin is able to synchronize the circadian activity of the fetal adrenal gland at gestational d 18. This day of gestation was chosen because at this period the fetal SCN clock is not yet functional (31), and the fetal pineal gland does not synthesize melatonin (32). They first demonstrated that the fetal rat adrenal, as in adults, is a functional circadian oscillator. Indeed it expresses daily (in vivo) and circadian (in vitro) oscillations of two major clock genes, *Per2* and *Bmal1*, but also the genes coding for steroidogenic acute regulatory protein (a key protein in corticosterone synthesis, regulated by the adrenal clock), early growth response protein 1 (an early transcription factor regulated by melatonin), and MT1. In vivo conditions, *Per2* and *Bmal1* mRNA levels oscillate in antiphase, as in the adult adrenal gland. However, in vitro conditions, although *Per2* and *Bmal1* genes still oscillate in a circadian manner, their phase relationship is modified, with their acrophases being 4 h apart instead of 9 h as in vivo. Strikingly, a 4-h melatonin pulse given during the subjective night restores the antiphasic pattern of both clock genes. The melatonin pulse also induced a phase shift in the circadian oscillations of MT1, early growth response protein 1, and steroidogenic acute regulatory protein mRNA levels. These data indicate that maternal melatonin can synchronize peripheral clocks within the fetus and therefore coordinate activity of several fetus among the same litter (Fig. 1). These findings reinforce the previous studies of the authors regarding the importance of maternal melatonin on fetal SCN and adrenal gland (33, 34).

In adults, the adrenal gland is also a peripheral circadian clock releasing glucocorticoids with a daily rhythm. Strikingly, the authors showed that the daily rhythm in fetal adrenal corticosterone content is almost opposite to the maternal circulating levels. This observation indicates that maternal and fetal adrenal clocks are synchronized independently and may serve different functions. In fetus, the adrenal gland is a key organ that orchestrates maturation processes via glucocorticoid production. Obviously, these processes require synchronization and this study indicates that maternal melatonin could constitute such a signal.

Now, considering the well-characterized physiological effect of melatonin as a synchronizer of annual functions with seasons, one could imagine that seasonal variations in circulating levels of maternal melatonin may impact on annual cycles in the developing fetus. Indeed, an earlier
study reported that the photoperiod under which hamster dams were raised or a paradigm of timed melatonin infusion in pinealectomized hamsters, resulted in after-effects on testicular development and body weight of the offspring (35). Furthermore, a recent study in mice reported that perinatal photoperiod has a strong and long-lasting effect on the molecular clockwork of the SCN as well as on neurobehavioral disorders (36). Whether this effect is mediated by melatonin is unknown, but it raises the hypothesis that seasonal imprinting during the perinatal period may contribute to subsequent depressive and anxiety-like behaviors.

Altogether these data indicate that newborn animals are sensitive to the photoperiodic history encountered during the prenatal period and that maternal melatonin may be the clock/calendar signal that primes the developing biology of the fetus during the perinatal period.

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