Mammalian pineal melatonin: A clock for all seasons

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Summary. The central role of the pineal gland and its hormone melatonin (MEL) in mammalian photoperiodic responses is discussed in terms of: 1) evidence for the involvement of MEL in photoperiodism, 2) which feature of the MEL secretion profile might be most important for regulating photoperiodic responses, 3) evidence for the modulation of responses to changes in daylength based on previous photoperiod exposure (i.e., photoperiodic history) and 4) how the MEL signal might be processed at its target sites to elicit physiological responses.
Key words. Melatonin; pineal gland; photoperiodism.

Pineal gland and photoperiodic responses

It has been firmly established that the pineal gland serves a central role in mammalian photoperiodism. Pinealectomy prevents photoperiodic responsiveness in a wide variety of eutherian and marsupial mammals[21]. Melatonin (MEL) appears to be the pineal product that mediates the role of the gland in photoperiodism[19], though several other biologically active indoleamines and peptides have been isolated from the mammalian pineal gland[43,45,46]. It has been difficult to establish the mechanism by which MEL conveys a daylength 'message' responsible for triggering photoperiodic responses, despite the abundant evidence for the central role of the hormone. The intent of this review is to: 1) summarize evidence for the involvement of MEL in photoperiodism, 2) discuss which feature of the MEL secretion profile might be most important for regulating photoperiodic responses, 3) review the evidence for the modulation of responses to changes in daylength based on previous photoperiod exposure (i.e., photoperiodic history) and 4) speculate on how the MEL signal might be processed at its target sites to elicit physiological responses.

Pineal and plasma MEL concentrations are low during the light phase and are elevated during a portion of the dark phase. Daylength change results in alterations in both the duration and amplitude of the nocturnal elevation of MEL[26], as well as the phase angle relationship between the MEL peak and various portions of the light:dark cycle. Thus, all three parameters – phase, amplitude and duration of the MEL peak – have received consideration as potentially critical variables for the physiological actions of MEL[3,8].

The preponderance of early information on the role of MEL in mammalian photoperiodism came from experiments employing constant release implants or timed daily injections of MEL, and most of this information was obtained in Syrian hamsters (*Mesocricetus auratus*). This species is a long-day breeder and exhibits gonadal regression following prolonged exposure to short days. Pinealectomy prevents these inhibitory effects.

Early reports indicated that administration of MEL via subcutaneous, continuous release implants had marked effects on the reproductive system in male Syrian hamsters. MEL implants induced testicular regression in long day-housed males[63], yet similar implants prevented testicular regression in hamsters transferred from long days to short days[49]. These observations suggest that MEL is involved in hamster photoperiodic responses, but they do not clarify the significance of the various features of the MEL secretion profile.

Daily injections of MEL can induce testicular regression in pineal-intact, long day-housed Syrian hamsters, but the timing of their administration is critical. Reproductive activity is inhibited only when MEL is administered during either of two phases of the circadian cycle – during a period of several hours just prior to lights-off and extending into the very early part of the night, or during a much briefer period at approximately the time of lights-on (dawn). However, pinealectomized (PINX) hamsters given single daily injections of MEL at these times do not exhibit gonadal regression. Collectively, these results suggest that the combined actions of exogenous and endogenous MEL might have been responsible for the gonadal inhibition observed in the pineal-intact, MEL-injected hamsters[62]. In support of this hypothesis, PINX hamsters given MEL injections three times daily over an interval of 6 h exhibit gonadal regression and, moreover, there is no apparent diurnal change in sensitivity to MEL in these animals[22,61]. In a more recent study, a circadian rhythm of sensitivity to single daily MEL injections was described in PINX hamsters, and this rhythm was most prominent when the animals were exposed to a short photoperiod[59]. The data from MEL injection and implant experiments fail to provide a clear indication as to whether the circadian phase of MEL secretion is important for its effects on reproductive status. Many of the data obtained from MEL-injection, pineal-intact animals readily lend themselves to the interpretation that the circadian phase at which the animal is exposed to MEL is critical, whereas most of the data from MEL-injected, PINX hamsters do not support such an interpretation.

Melatonin target sites

It is generally thought that MEL affects reproductive status primarily by acting at one or more sites in the central nervous system, rather than by direct actions

at the level of the pituitary gland or the gonads[19]. It has recently been found that radiolabeled MEL binds to the suprachiasmatic nuclei (SCN), the median eminence/pars tuberalis and several other brain sites in a variety of species, including Syrian and Siberian hamsters[52, 65, 67, 71, 73, 74] (see Morgan and Williams, this issue). Some data support the involvement of the SCN in the reception of the MEL signal. In white-footed mice, constant-release MEL implants in the anterior hypothalamus/SCN region lead to inhibition of reproductive activity[18]. In the Syrian hamster, melatonin implants located in this region block the inhibitory effects of short photoperiods[25]. Daily application of MEL to the anterior hypothalamus/SCN for 10 h/day also resulted in short day-like gonadal regression in long day-housed, PINX white-footed mice. This was accomplished via timed insertion and removal of MEL-filled cannulae into chronically implanted guide cannulae[12]. Finally, we have preliminary results that further suggest that the SCN may be a target site for receiving the MEL signal in Siberian hamsters. Long day-housed, PINX male hamsters received either control or bilateral lesions of the SCN and subsequently were given long (10 h) or short (5 h) daily MEL infusions. Lesions of the SCN disrupted locomotor activity and drinking rhythms, and blocked the effects of long duration MEL infusions (Bartness, Goldman and Bittman, unpublished observations). These results lend further support for a possible role of the SCN in the reception of the MEL signal that triggers photoperiodic responses. Since some circadian rhythms were eliminated by the SCN lesions, these data suggest some involvement of the circadian system may be required for appropriate responses to MEL signals.

Melatonin pulse duration

Administration of MEL by daily injections provides a means for controlling the circadian phase of MEL exposure; however, this method does not permit the precise control of the duration of exposure to each MEL pulse. Both the circadian phase and the duration of exposure to MEL can be independently varied and precisely controlled by employing timed daily infusions of MEL. The reproductive response of PINX male Siberian hamsters (*Phodopus sungorus*) has been examined using this paradigm and found to be critically dependent upon the duration of the hormone infusion. In prepubertal males, 8-h or longer MEL infusions were inhibitory to testis growth (i.e., a short day-type response), whereas infusions of 6 h or less were stimulatory (i.e., a long day-type response)[8, 9]. In adult males, daily MEL infusions of 10 h or 12 h duration led to testicular regression whereas infusions of 4–6 h duration were stimulatory to testis growth[2, 3, 15]. In these experiments, the time of day at which the MEL infusions were administered did not significantly influence the nature of the reproductive response; infusions administered during the light phase were just as effective as those given at night. In adult males, changes in body weight and lipid metabolism also were influenced by the duration, but not by the circadian phase of daily infusions of MEL[2, 3]. Recently, a timed infusion paradigm was used in Syrian hamsters with results similar to those observed in Siberian hamsters; that is, long, but not short, duration MEL infusions resulted in gonadal regression in PINX males[40].

It is important to emphasize that the MEL infusion studies described above were carried out in PINX hamsters. In pineal-intact, long day-housed Siberian hamsters daily short duration MEL infusions (i.e., 5 h) inhibited testis growth in a phase-dependent manner. Thus, gonadal regression only occurred when the MEL infusion was initiated shortly before the beginning or end of the endogenous secretion of the hormone so as to overlap with, and extend, the duration of elevated circulating MEL[20]. These data indicate that the extension of the duration of elevated circulating MEL might be the process underlying the short day-type responses that result from daily MEL injections given shortly before, or after, the endogenous nocturnal MEL peak in long day-housed, pineal-intact hamsters.

A series of studies in sheep employed the daily, timed MEL infusions paradigm similar to that used in hamsters. The comparison of results in sheep to those in hamsters is particularly interesting since reproductive activity in sheep is stimulated by exposure to short, rather than long, daylengths[33]. Daily MEL infusions in PINX ewes elicited reproductive responses similar to those that are typically under photoperiodic control. As with the studies in Siberian hamsters, long duration MEL infusions elicited a short day-type response, in this case stimulation of luteinizing hormone secretion, and short duration MEL infusions led to a long day-type response[5, 35, 75]. The effect of the MEL infusion was the same whether it was administered during the day or the night[69].

Although the results of the MEL infusion experiments in hamsters and sheep strongly suggest that the duration of the nocturnal MEL pulse is the feature of the MEL profile that is important for mediating photoperiodic responses, it remains possible that the MEL rhythm itself entrains a circadian rhythm of MEL sensitivity[58]. In the experiments discussed above, MEL infusions were repeatedly administered at the same time of day in each experimental group, thereby possibly establishing a rhythm of sensitivity to the hormone during the first few days of infusion. Additional experiments in Siberian hamsters have yielded data relevant to this possibility. In one study, PINX hamsters were given long (12-h) or short (6-h) duration MEL infusions at non-24-h intervals while the animals were housed in continuous darkness. Males receiving long duration MEL infusions at intervals of 24.33 or 24.78 h had regressed testes, whereas those receiving short duration MEL infusions at the same intervals failed to exhibit regression (Darrow and Gold-

man, unpublished data)[11]. These results add further support to the hypothesis that the duration of each MEL pulse, rather than its circadian timing, is important for determining the reproductive response. In another study, PINX Siberian hamsters were given MEL infusions at intervals of 18, 24, 36 or 48 h. Males receiving short duration (6-h) MEL infusions at any of these intervals showed no indication of reproductive inhibition, whereas males receiving long duration (10-h) infusions at 18- or 24-h intervals had regressed gonads. It should be noted that regression was more complete in the males given long duration MEL infusions at 24-h intervals relative to those experiencing an 18-h infusion cycle. Testes size was not decreased in hamsters receiving long duration MEL infusions at 36- or 48-h intervals suggesting that hamsters must experience long duration MEL pulses more frequently than once every 36 h for reproductive inhibition to occur. It remains unclear whether an entraining action of MEL is involved in the lesser degree of testis inhibition in 18-h versus 24-h infusion cycles[15]. Essentially similar effects occur in the Syrian hamster, where 10-h infusions of MEL delivered every 25, 24, 23 or 20 h induce gonadal atrophy, whereas infusions given every 28 h or 48 h fail to do so[40].

Importance of continuity of MEL pulse

The importance of the continuity of each MEL pulse signal has been demonstrated using the timed, daily MEL infusion paradigm in Siberian hamsters. In the initial studies, two daily MEL infusions given to prepubertal Siberian hamsters did not have additive effects when separated from each other by a period of 2 or 3 h. For example, two daily 5-h MEL infusions did not inhibit testis growth when the two infusions were separated by an interval of 2 h in these animals, whereas single daily MEL infusions of 8 h or greater inhibited testis development. When separated by only 1 h, the two infusions showed a partial additive effect[20]. Similar results were found in adult Siberian hamsters receiving two 5-h MEL infusions separated by 2 h without infusion. In this study both the reproductive and body weight responses were dependent upon the continuity of the long duration MEL infusions[8]. Direct application of MEL to the anterior hypothalamus/SCN region in white-footed mice (see above) also demonstrates the importance of the continuity of the MEL stimulation[12]. Thus, reproductive inhibition was observed when the implants were left in place for a continuous 10-h period each day, but not when the implants were presented for two 5-h sessions each day, separated by a period of 3 h without MEL[12]. Together, these results suggest that it is not merely the total daily exposure to MEL that is important; rather, MEL must be present relatively continuously over some critical time span to elicit a short day-type response. The ability of the animal clearly to distinguish separate MEL pulses might represent a part of a mechanism designed to measure accurately the duration of each nightly period of MEL secretion. Thus, the system that 'measures' the duration of the MEL pulse must be 'reset' soon after the termination of a pulse so as to be capable of accurately measuring the pulse that will occur on the next night[20].

Species differences in response to pinealectomy

Initially, it was believed that the pineal gland functions primarily to inhibit reproduction in mammals. This idea was largely based on the observations that pinealectomy prevented the inhibitory effect of exposure to short photoperiods on the reproductive system in Syrian hamsters[47]. Data from other species have led to a revision of this hypothesis of pineal function. For example, pinealectomy in Turkish hamsters (*Mesocricetus brandti*) actually *induces* testicular regression in animals exposed to a stimulatory daylength[10]. A similar observation was made for European hamsters, *Cricetus cricetus*[39]. These species-differences in response to pinealectomy might seem surprising since Syrian, Turkish and European hamsters are all long-day breeders and because Turkish hamsters are so closely related to Syrian hamsters. However, it may be possible to explain the differences in the context of the hypothesis of MEL function discussed above. To illustrate this point, it should be noted that male Turkish hamsters maintain large testes when housed under a 16L photoperiod, but that testicular regression occurs when the daylength falls to 14L or when it increases to 18L or more. In addition, the duration of the nocturnal peak of pineal MEL decreases progressively as daylength increases in this species[28]. It may be that in male Turkish hamsters only a narrow range of MEL pulse durations – for example, only those corresponding to the MEL pattern that occurs in 16L – stimulate reproductive activity and that longer or shorter pulse durations inhibit reproduction. This could explain why either pinealectomy or administration of MEL by daily injections[10,29] can result in testicular regression in this species.

Photoperiodic history and the MEL system

Experiments designed to examine the role of photoperiodic history in determining responses to a particular photoperiod indicate that photoperiodic history may have a significant role in determining how an animal will respond to MEL. In Syrian hamsters and other long day-breeding, photoperiodic rodents, the reproductive system does not remain permanently inhibited when animals are held continuously under short days; rather, after several months the animals become 'photorefractory' and reproductive function is restored[33]. Hence, the inhibitory effect of short days is no longer present during the refractory phase of the annual cycle. In some species, refractoriness may also occur after long-term exposure to long days. For example, reproductive activity is initially

inhibited by exposure to long days in ewes, but this inhibition is dissipated after several months of continued long day exposure [54]. Ewes also become refractory to the stimulatory effect of short days after prolonged exposure to winter daylengths [53]. Photorefractoriness is associated with a parallel 'refractoriness' to MEL as demonstrated by the loss of the reproductive response to the MEL signal during seasonal reproductive transitions in ewes [34]. In addition, exogenous MEL is not capable of inhibiting reproductive activity in photorefractory Syrian hamsters, just as short day exposure no longer is inhibitory at this time [4, 48, 68]. In Syrian hamsters, refractoriness to short days can be reversed by exposing animals to long days for several weeks [60], and under these conditions, refractoriness to the effects of MEL are also reversed [48]. Indirect evidence suggests that the pineal gland is involved in the breaking of refractoriness by long day exposure [6]. Therefore, it seems probable that short duration MEL peaks conveying a 'long day signal' are responsible for terminating the photorefractory state in hamsters.

A second type of photoperiodic history effect was demonstrated several years ago in Japanese quail. In this species, a given photoperiod can be either stimulatory or inhibitory to reproductive activity depending on whether the birds had previously been exposed to a longer or shorter daylength; thus, increasing daylengths stimulate and decreasing daylengths inhibit reproductive status [17]. Early experiments did not reveal a similar phenomenon in Syrian hamsters. For example, daylengths of 12.5 h or greater were stimulatory to reproduction whereas shorter daylengths were inhibitory, regardless of whether the hamsters had previously been exposed to very long or very short photoperiods [14]. However, more recent studies in other rodents and in sheep have revealed photoperiodic history effects similar to those reported for Japanese quail. The first reports of such effects in mammals involved photoperiod cues received pre- and postnatally in montane voles and Siberian hamsters during gestation. Exposure to a 14L:10D photoperiod (14 h of light and 10 h of darkness) after birth can either stimulate or inhibit early testicular development of the male pups depending upon the photoperiod experienced by the mother during pregnancy. When mothers are exposed to daylengths of 14L or less during gestation, their pups showed rapid gonadal development in a postnatal photoperiod of 14L; when the prenatal photoperiod is 16L, postnatal testis growth in 14L is slower [30, 57]. Cross-fostering experiments in voles and hamsters indicate that a photoperiodic 'message' is transmitted from the mother to her fetuses prior to birth (Elliott and Goldman, unpublished observations) [31, 51]. Further studies in Siberian hamsters showed that the mother's pineal MEL is somehow involved in the transmission of the photoperiodic message to her pups (Elliott and Goldman, unpublished observations), that the message is related to the duration of nightly MEL secretion by the mother [72], and that the transfer of information occurs during late gestation [70]. Since MEL crosses the placenta [50, 76, 77], it is tempting to speculate that maternally derived MEL may directly convey a daylength message to the fetuses. As with the situation in quail, the end result of this system is that offspring born into an environment where daylength is increasing ('spring') will exhibit relatively rapid reproductive development, while gonadal growth may be held in abeyance for offspring born into decreasing photoperiods ('fall').

Various photoperiodic history effects have been described in adult Siberian hamsters and sheep. For example, reproductive activity can be induced by a sudden switch from long days to an unchanging short day photoperiod in ewes, and this activity will generally be maintained for 2–3 months before photorefractoriness develops and reproductive activity ceases. The reproductive response to this artificial photoperiod change is temporally different from the response to natural photoperiods during the fall/winter; under natural photoperiods the reproductive phase persists for 5–6 months [35, 38]. Evidence indicates that the progressively decreasing daylengths encountered under natural photoperiod conditions may be responsible for the prolongation of the breeding season in ewes. Thus, ewes subjected to a single-step decrease in photoperiod from 16L to 8L or from 16L to 12L showed periods of increased LH secretion lasting for approximately 50–60 days. However, when the daylength was decreased in two stages – first from 16L to 12L and then, 55 days later, to 8L – the period of increased LH activity lasted almost twice as long [37].

A somewhat different type of photoperiod history effect may prevail in Siberian hamsters. Male hamsters transferred from 16L to 10L exhibit gonadal regression within 8 weeks and undergo spontaneous recrudescence after approximately 24–26 weeks of short day exposure (Goldman, unpublished data) [13]. When hamsters are transferred from 16L to 14L, testicular regression is apparent at 8–12 weeks, and by 16 weeks gonadal recrudescence is well underway [13]. There are at least two possible explanations for the earlier recrudescence in 14L as compared to 10L. First, it may be that the timing of spontaneous recrudescence is different in 14L as compared to 10L. Since spontaneous recrudescence appears to be triggered by an endogenous 'seasonal' timing mechanism, this explanation would imply that the input to this timing mechanism distinguishes between 14L and 10L, even though both photoperiods are inhibitory to the reproductive system. This would be somewhat different from the commonly held view that the timing of spontaneous gonadal recrudescence is relatively independent of the exact duration of the inhibitory photoperiod [16]. The second explanation involves the hypothesis that the earlier testicular recrudescence in the 14L male hamsters may not have involved the same 'spontaneous' recrudescence timing mechanism operative in the 10L animals. Instead, it may be that after prolonged exposure to 14L – or

perhaps to any sufficiently short photoperiod – a downward shift occurs in the daylength required for stimulation of reproductive activity so that 14L becomes a stimulatory photoperiod. With this hypothesis, the time course of testicular changes in 14L would result from changes in the reproductive response to this photoperiod, with gonadal recrudescence being a result of photostimulation rather than occurring through a photoperiod-independent mechanism. A very similar hypothesis has been proposed to explain why European starlings exhibit circannual rhythms of reproductive activity when held under a constant 12L:12D photoperiod, but not when kept under longer or shorter daylengths. It has been suggested that 12L can be alternately stimulatory or inhibitory to the reproductive system in starlings [23, 24, 56].

The second possibility listed above should be considered in the design and interpretation of studies of photoperiodic history effects. For example, in one experiment, male Siberian hamsters were transferred from 16L to 8L at 21 days of age and remained in 8L until age 3.5–5 months. At this time, some of the 8L males, along with animals of the same age raised in 16L, were transferred to 14L. After an additional 10 weeks, the animals transferred from 8L to 14L had large testes, while the males remaining in 8L still had regressed testes. Hamsters transferred directly from 16L to 14L had small testes after 10 weeks. This experiment demonstrated that testicular size after 10 weeks in 14L is different depending on whether the animals had previously been housed in 16L or 8L [27]. While it is tempting to conclude that the animals were responding to the direction of change in the photoperiod, there were no controls left in 14L from the beginning of the experiment (i.e., transferred from 16L to 14L at this time). If such a group had been included, it is possible that their testes would have regressed initially, but then may have exhibited recrudescence by the end of the study. One might then invoke the second hypothesis presented above as a possible explanation. That is, a downward shift in the critical photoperiod might occur following several weeks exposure to either 8L or 14L, with the result that 14L becomes a stimulatory photoperiod in both cases.

Despite the effects of photoperiodic history on overt reproductive responses, the evidence available to date suggests that the duration of nocturnal elevation of pineal and serum MEL reflects the prevailing daylength and is relatively independent of photoperiodic history. For example, in Syrian hamsters the pattern of day/night changes in pineal MEL content is similar in photosensitive and photorefractory animals when they are exposed to the same daylength [55]. In sheep, one report indicates changes in the circulating MEL pattern associated with photorefractoriness [1], but two later studies indicate no such difference between MEL patterns of photosensitive and photorefractory ewes [37, 38]. Furthermore, species or subspecies that exhibit little or no effects of daylength or MEL on reproductive activity, still exhibit MEL patterns that varied with daylength in a manner similar to that described for MEL-responsive species [32, 36, 64, 66]. It remains possible that non-reproductive parameters are influenced by the MEL pattern in these species [7, 41, 42].

The intricate effects of photoperiodic history on the response to a given daylength and/or to a given MEL signal require a reevaluation of the importance of the duration of nightly MEL secretion. A working hypothesis might be formulated whereby the information content of each nocturnal MEL pulse is encoded in its duration. However, it is apparent that mammals possess a great deal of flexibility with respect to downstream processing and responding to this signal. One factor that influences the response to a MEL pulse of a given duration is the nature of the previous MEL patterns experienced by the animal, indicative of the photoperiodic history. In addition, it seems virtually certain that a variety of other factors such as temperature, food, water and social stimuli will also be found to interact with the daylength information provided by MEL signals, just as these factors can influence the response to daylength itself. It has been proposed that the annual progression of changes in MEL pulse duration, reflecting photoperiodic change, may serve as the primary source of environmental information for a sort of internal 'calendar'. Such a calendar might provide the organism with a continuous reference to the time-of-the-year that could be consulted in the process of determining responses to a variety of biologically relevant stimuli [21]. If the daily changes in the pattern of pineal MEL secretion do, in fact, serve primarily to maintain synchrony between an endogenous 'calendar' and local annual time as determined by daylength, then it may be somewhat misleading to refer to the actions of MEL on reproductive activity. Rather, it might be more accurate to think of MEL as the major endocrine input to a calendar system that serves as a reference for the timing of annual reproductive rhythms and other seasonal changes [19].

Measurement of MEL pulse duration: Speculations

It seems appropriate to ask what biological mechanism might be employed to 'measure' the duration of the nocturnal elevation of plasma MEL in the context of the hypothesis for the action of MEL that has been presented here. The precision of the MEL duration measuring system is exemplified by the data from Siberian hamsters showing that these animals are capable of discriminating rather small differences in MEL pulse duration [8]. In studies of the mechanism of action of other hormones, little attention has been given to investigating the extent to which the duration of exposure to the hormone is important in determining the overt actions elicited by the endocrine stimulus. However, interesting temporal relationships have been reported for the action of estradiol in eliciting female sexual behavior. Female rats generally begin to exhibit lordosis approximately one day follow-

ing exposure to continuously elevated levels of estrogen. More detailed studies revealed that a comparable response could be obtained following two separate 1-h periods of exposure to estradiol, provided the second exposure began not less that 4 h nor more than 13 h after the end of the first treatment[44]. It seems reasonable to believe that many hormone actions might involve certain critical temporal constraints; for example, duration of exposure to elevated levels of the hormone. The MEL response system may simply represent a special case whereby the temporal aspects of the hormone's action have been refined to provide the animal with the ability to make fine discriminations between MEL pulses of slightly different durations.

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