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The Effect of Daily Injections and Constant Release Implants of Melatonin on the Endogenous Pineal Melatonin Rhythm in Golden Hamsters[1] (41713)

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Abstract. In this study we tested the hypothesis that exogenous melatonin exerts its effects on the reproductive system of hamsters by directly or indirectly altering the endogenous rhythm of melatonin production and release. Melatonin was injected in male hamsters housed on LD 14:10 (lights 0600–2000 hr) either at 1200 or 1900 hr (15 μg in 0.1 ml ethanol:saline 1:10) daily for 12 weeks. Testicular regression occurred in all animals of the 1900-hr injection group, while melatonin injected at noon was without effect. A third group of animals received small implants of melatonin subcutaneously at 0, 4, and 8 weeks. Implants were 4 mm in length and contained a melatonin:beeswax mixture (1:25) drawn up into polyethylene tubing (2.2 mm i.d.). These implants release approximately 10–15 μg melatonin/day, and had no effect on testicular size, as these animals also remained on LD 14:10. After 12 weeks the animals of each group were sacrificed at 1- or 2-hr intervals around the clock. Pineals were saved and assayed for melatonin content. In each group the nocturnal rhythm of pineal melatonin was similar; peak melatonin levels were achieved 6 hr after lights out (0200 hr) and levels remained elevated for approximately 4 hr. These results exclude a mode of action of exogenous melatonin on the pineal melatonin rhythm as a basis for the testicular response to melatonin in hamsters. They also pose some interesting questions of feedback regulation by melatonin on its own production and release.

The golden hamster is a seasonally breeding rodent, with reproductive activity confined to the spring and summer months. The environmental parameter employed by this species to time reproduction appears to be photoperiod (1). Although the means by which photoperiod accomplishes this have not been elucidated in full, certain parts of the control system have been identified. The photoreceptors are retinal (2), and transmit light information directly to the suprachiasmatic nuclei (SCN) of the hypothalamus via a retinohypothalamic projection (3). The SCN is an endogenous oscillator, or clock, and its neural activity is entrained by the ambient photoperiod. In the absence of the SCN the hamster is aphotoperiodic; it cannot "read" photoperiod and its clock-driven rhythms are also aperiodic (4, 5). One of the rhythms driven by the SCN is the daily rhythm of pineal melatonin content and release (6). Melatonin concentrations increase during darkness and are minimal during the day throughout the annual reproductive cycle of the hamster (7). Abolition of this rhythm renders the animal aphotoperiodic; this has been accomplished by SCN lesion, interruption of the sympathetic innervation of the pineal and by pinealectomy (2, 8). Thus daily output of pineal melatonin is important in the animal's capacity to read photoperiod and thereby control annual bouts of reproductive activity.

Melatonin administration to intact hamsters has yielded provocative effects on the reproductive system. Properly timed daily injections of melatonin cause testicular regression or induce anestrus (9), while injections at other times of the day are without effect. Constant release implants (subcutaneous) of melatonin, in addition to causing alterations in hamster pineal gland morphology (10), prevent photoinduced testicular regression on short days and growth on long days (11), but have no effect on spontaneous testicular development in prepubertal and adult (12) animals. The mechanisms by which melatonin generates these responses remain unknown. One possible mode of action of exogenous melatonin is to function by some direct or

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indirect feedback mechanism to alter the endogenous rhythm of pineal melatonin production and release. To test this possibility we examine here the effects of daily injections of melatonin at times known to be effective (1900 hr) and ineffective (1200 hr) in causing testicular regression, and of constant-release melatonin implants on the endogenous rhythm of pineal melatonin production and release.

Methods. Adult male hamsters raised from birth in our lab on a photoperiod of LD 14:10 (lights 0600–2000 hr) were used in this investigation. They were all born within 4 days of one another and placed in the experiment at 12 weeks of age. Food and water were continually available.

Three groups of 120 animals each were selected at random. Group 1 received daily melatonin injections at 1200 hr, Group 2 received injections at 1900 hr daily, and Group 3 received small implants of melatonin in the nape of the neck at 0, 4, and 8 weeks of the investigation. Melatonin injections were 15 μg/0.1 ml ethanol:saline (1:10), subcutaneously. Melatonin implants consisted of a melatonin:beeswax mixture (1:25) in polyethylene tubing (2.2 mm i.d.) of 4 mm length. Implants are open-ended and release approximately 10 μg melatonin/day as measured by reduction with time *in vivo* of implant melatonin content.

After 12 weeks of treatment the animals were sacrificed by decapitation at 1- or 2-hr intervals around the clock. The two light:dark transition groups (2000, 0600 hr) along with seven other groups were killed in the dark under a red working lamp. Testes were removed and weighed. Pineals were removed and immediately placed in 1 ml of ice-cold PBS (0.1 *M*, pH 7.2). The glands were then homogenized and assayed in duplicate by RIA for melatonin content (13). Results are expressed as picograms melatonin per gland.

Results. The effect of melatonin injections and implants on testicular weight is illustrated in Fig. 1. Testicular regression occurred in the 1900-hr injection group only. As can be seen in Fig. 2, the pineal melatonin rhythm in the affected and nonaffected groups were strikingly similar. All displayed peak concentrations 6 hr after lights out (0200 hr), with concentrations elevated until 0500 hr. Pineal melatonin content in all groups remained very low during the hours of light.

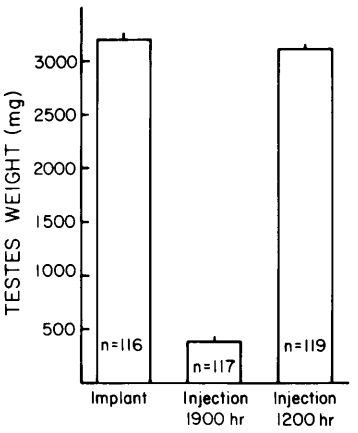

FIG. 1. Testes weight in the three experimental groups. Each bar represents the mean ± SEM (vertical line) testes weight for the indicated group. The number of animals per group is indicated in each histogram.

Discussion. In designing this investigation, our initial thoughts centered on the possibility that the reported effects of exogenous melatonin on reproductive activity in golden hamsters might be due to feedback action by melatonin on its rate of production and/or release or the timing thereof. We reasoned that melatonin might feed back directly on the pineal or indirectly via the circadian system responsible for driving the endogenous melatonin rhythm. A previous investigation of this possibility (14) had shown that neither the time of the initial increase in pineal melatonin nor the magnitude of the increase was altered by daily melatonin injections. These authors, however, found that melatonin concentrations were still elevated at lights on in those hamsters receiving evening melatonin injections, whereas in control hamsters and those receiving ineffective morning injections, melatonin concentrations had decayed to basal levels before the hamsters were exposed to light. The appearance of melatonin at lights on in hamsters responding to melatonin injections may have been important and warranted repeating. Our findings suggest that this effect of evening injections is not a consistent finding, thus we agree with Tamarkin *et al.* (14) that it appears that melatonin injections do not induce gonadal regression in Syrian

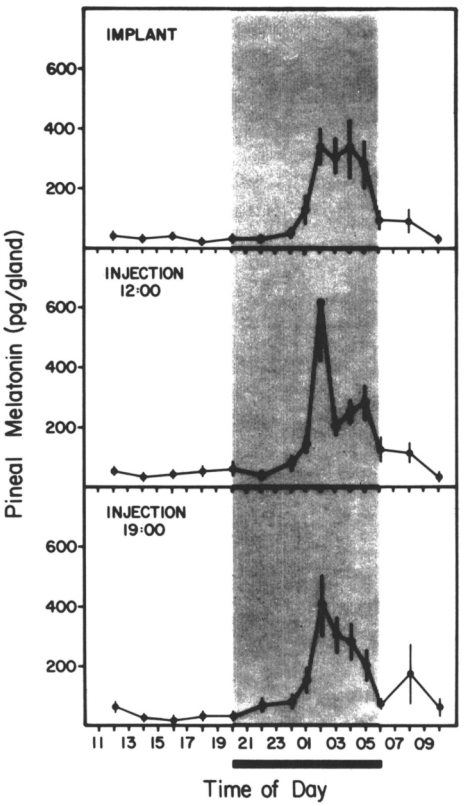

FIG. 2. Pineal melatonin content (pg melatonin/gland) in the three experimental groups. Each point represents the mean value of seven to eight glands. Vertical lines represent SEM. Shaded area and black bar represent the hours of darkness.

hamsters by causing major alterations in the daily pattern of pineal melatonin content.

We must therefore examine alternative hypotheses of the mode of action of melatonin. Lynch and colleagues (15, 16) have elegantly shown melatonin to cause testicular regression in *Peromyscus* when small melatonin:beeswax pellets are implanted in or near the SCN. We have duplicated these results in golden hamsters (Hubbard and Stetson, unpublished) and extended them to show that effective brain implants are without effect on the circadian system in animals held on either LD 14:10 or LD 6:18. Thus, if the SCN is a target tissue for exogenous (and endogenous) melatonin, the target cells of the nuclei appear to be other than those constituting the circadian oscillator that drives the locomotor activity and pineal melatonin rhythms in golden hamsters. Similarly, we have shown in adult females rendered anestrous by properly timed melatonin injections (17) that the daily clock-timed rhythm of LH and FSH release is not altered in magnitude or phase. Indeed, the SCN of rodents has been shown, by histochemical and histoimmunological criteria, to be an exceedingly complex structure (18), and it is likely that different cell types may control different peripheral and/or internal functions.

Other possible target tissues for melatonin may be the anterior hypothalamus, arcuate region, or perhaps even extrahypothalamic neural regions. Very few investigations have been published to date addressing these and other potential melatonin targets. Reiter and colleagues have suggestive evidence for a target outside of the medial basal hypothalamus (19) for melatonin's effect on the reproductive system in male hamsters. This is based on the response to exogenous melatonin in animals bearing hypothalamic knife cuts, and suggests that target cells (neurons) enter the MBH from an anterior direction. These studies must be expanded before the particular neurons in question can be identified.

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