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Melatonin Prevents Refractoriness to Short Days in Male Hamsters[1] (40204)

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Exposure to short daylengths (less than 12.5 hr of light per day) produces testicular regression in golden hamsters (1). Regression is dependent upon the pineal gland and is complete within 10 weeks of transfer to short days (2). Regrowth of the gonads occurs spontaneously in hamsters remaining in short days; it can be accelerated by exposing the hamsters to long photoperiods (3). In either case recrudescence is followed by unresponsiveness to short photoperiods, i.e., the gonads remain large and functional despite continued exposure to short days.

Subcutaneous implants of the putative pineal hormone melatonin can prevent short photoperiods from inducing testicular regression in hamsters (4, 5). Three questions concerning this "progonadal effect" of melatonin were addressed in the present study. (1) Do tonic levels of melatonin prevent regression by rendering animals refractory to the effects of short days? (2) Do melatonin implants "trigger" a long-term resistance to the regressive effect of short days? (3) Do melatonin treatments which block testicular regression in short days also prevent subsequent refractoriness to short days?

Materials and methods. Sexually mature golden hamsters (*Mesocricetus auratus*) were obtained from the Lakeview Hamster Colony (Newfield, NJ) or were born in our laboratory from similar stock. Animals were group housed in LD 14:10 (lights on 2000 PST) prior to each experiment. Room temperature was maintained at 21° and animals had *ad libitum* access to water and food (Simonsen rat pellets). Surgery was performed under sodium pentobarbital anesthesia (80 mg/kg of body weight) or under ether anesthesia.

Testicular condition was monitored by periodic laparotomies (6). The product of the maximum length and width of the testis was divided by the body weight to give a reliable index of relative testicular weight. This testis index (TI) reflects spermatogenic and steroidogenic activity (7). A TI above 1.8 indicates full reproductive competence while one under 1.0 reflects complete testicular collapse. This technique permits assessment of gonadal condition in individual hamsters at multiple time points.

The first series of experiments (see Table I) was initiated in October of 1975; hamsters were implanted with beeswax pellets prepared by the methods of Reiter *et al.* (4). Nine animals were implanted at weekly intervals with pellets composed of 1 mg melatonin (Regis Chemical Co.) and 24 mg of beeswax; nine controls were implanted weekly with pellets containing beeswax alone. In a second experiment, hamsters either received a single implantation of two 50 mm Silastic pellets (Dow Corning Corp., cat. no. 602-235, 1.47 mm i.d., 1.96 mm o.d.) filled with 172 mg melatonin or two empty pellets.

All hamsters were transferred from the LD 14:10 to the LD 2:22 photoperiod (lights on 0800) at the time of the first pellet implantations and remained there for the duration of the study. Laparotomies were performed at the time of transfer and 10 weeks later (beeswax-pellet implants) or 8.5 weeks later (Silastic implants). Pellets were removed after this laparotomy. The Silastic pellets were dried and weighed by the method of Turek *et al.* (5). Hamsters which had been implanted with beeswax were laparotomized again 10 and 18.5 weeks after the removal of the pellets; those implanted with Silastic were laparotomized 8 and 21.5 weeks following capsule removal.

Additional experiments were conducted in which hamsters from LD 14:10 were trans-

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TABLE I. SUMMARY OF EXPERIMENTAL TREATMENTS.

Experiment No.	Vehicle	Photoperiod	Laparotomy times
1A	beeswax	LD 2:22 throughout	weeks 0,[a] 10,[b] 20, 28.5
1B	Silastic	LD 2:22 throughout	weeks 0,[a] 8.5,[b] 16.5, 30
2	Silastic	LD 6:18 weeks 0–9 weeks 19–27 LD 14:10 weeks 9–19	weeks 0,[a] 9,[b] 19, 27

[a] Capsules implanted at time of laparotomy.
[b] Capsules removed at time of laparotomy.

ferred to LD 6:18 (lights on 0400). At the time of transfer (week 0), animals were laparotomized and implanted with blank (n = 8) or melatonin-filled (n = 10) Silastic capsules. All pellets were removed, dried and weighed at the end of week 9. At this time a second laparotomy was performed and the original LD 14:10 photoperiod reinstated. After 10 weeks the hamsters were again laparotomized and returned to the original LD 6:18 photoperiod. Final laparotomies were performed 8 weeks later.

Results. Complete testicular regression occurred in blank-implanted hamsters within 8.5–10 weeks of their transfer to the LD 2:22 photoperiod ($P < 0.01$ vs. initial TI; Fig. 1a and b). The results of beeswax and Silastic methods of administering melatonin were essentially identical. In confirmation of the reports of Reiter *et al.* (4) and Turek *et al.* (5), melatonin completely prevented testicular regression in most hamsters ($P < 0.01$ vs. blank implants; no significant regression from initial TI). Silastic implants were 3.6 ± 0.5 mg lighter at the time of their removal, suggesting an average melatonin release rate of about 60 μg per day.

Removal of melatonin pellets resulted in testicular regression with a time course similar to that observed in controls upon initial transfer to short days. Spontaneous recrudescence, again with a normal time course, occurred in hamsters remaining in LD 2:22.

Melatonin also prevented testicular regression in the LD 6:18 photoperiod (Fig. 2). Although there was a significant decrease in TI in melatonin-treated animals ($P < 0.001$ vs. pretreatment TIs), regression was marginal and testes did not regress as completely as did those of blank-implanted controls (TI's of 1.71 ± 0.09 and 0.66 ± 0.08 respectively; $P < 0.001$). Following capsule removal, the 10 weeks of long day exposure was effective

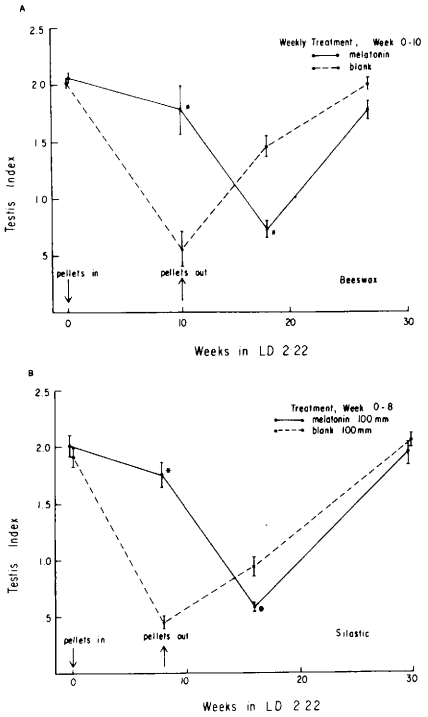

FIG. 1. Testicular indices of hamsters administered (A) melatonin in beeswax vehicle and (B) melatonin in Silastic capsules. In each case implants remained in place from the time of initial laparotomy (week 0) until the second laparotomy was performed. Asterisks indicate values for melatonin and blank-implanted groups differed significantly at the time of laparotomy ($P < 0.01$).

in completely recrudescing the testes of control hamsters. Upon return to short days, the testes which had previously been prevented from regressing by melatonin implants completely regressed ($P < 0.001$ vs. previous TI's). Hamsters which had borne blank capsules and whose testes had previously regressed were not uniformly insensitive to the effects of short days; 4/8 surviving hamsters underwent marginal (n = 2) or complete (n = 2) second regression. Testicular regression

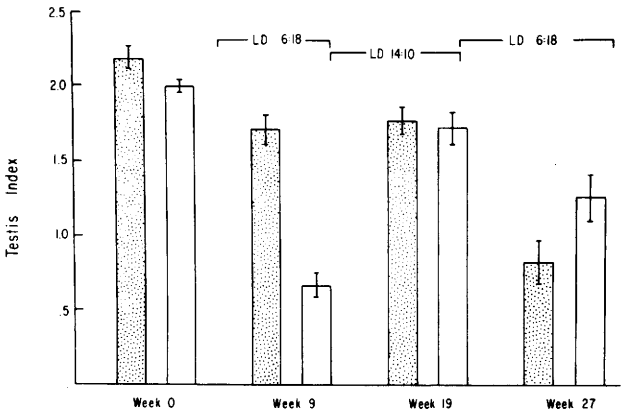

FIG. 2. Testicular indices of hamsters in the second experiment. Photoperiodic conditions as indicated; capsules removed after 9 weeks of short days. Stippled bars represent melatonin-implanted hamsters; open bars indicate blank-implanted controls. Values for melatonin- and blank-implanted animals differed significantly at week 9 ($P < 0.01$ but not at week 27 ($P = 0.07$).

among formerly melatonin-implanted hamsters was more pronounced than that in controls ($P = 0.07$).

Discussion. The results suggest that melatonin's "progonadal" effects are not attributable to the hamster's insensitivity to short days. Nor is melatonin able to "trigger" a long-term resistance to the induction of regression by short days; protection of the gonads lasts only as long as melatonin capsules are in place and regression follows quickly once the capsules are removed. In all respects hamsters which had borne melatonin capsules responded as though they had not been exposed to short days until those capsules were removed.

In the present experiment, exposure to short days in the presence of melatonin completely eliminated the refractoriness to short photoperiods observed in 75% of blank-implanted hamsters. Zucker and Morin (8) found that some individuals whose testes did not regress during their initial 6 week exposure to short days were refractory when subjected to a subsequent 9-week short day challenge. They concluded that testicular regression is unnecessary for the induction of refractoriness. It remains possible that the initial exposure to short days may have induced changes in the brain, pineal, and/or pituitary which were not immediately reflected by alterations in testicular size. The present results support the hypothesis that neuroendocrine changes associated with regression, and not exposure to short days *per se,* are necessary for the induction of testicular refractoriness.

It is unlikely that either consequence of short day exposure is due to an action of melatonin on the gonads (9, 10). Chronic melatonin administration may prevent both regression and the subsequent development of insensitivity to short days by affecting common neuroendocrine mechanisms. Endogenous melatonin levels undergo circadian fluctuations (11, 12) and the timing of peak melatonin concentrations determines whether regression will occur (13, 14). While melatonin implants do not disrupt the general circadian system (as assayed by locomotor activity; Bittman, unpublished observations), the relatively constant release pattern characteristic of such pellets might interfere with the utilization of such rhythms for the measurement of daylength by the pineal and/or its target(s).

Alternatively, exogenous melatonin might act on the pineal to prevent release of an antigonadotrophin. Such a feedback action of melatonin would account for the prevention of both regression and insensitivity to short days, regardless of whether refractoriness normally results from pineal exhaustion or hypothalamic insensitivity to a pineal hormone (15).

Many hamsters in whom gonadal recrudescence was accelerated by 10 weeks of long day stimulation were not refractory to a subsequent short-day challenge. This is consist-

ent with the finding that 10 weeks of long-day exposure terminates refractoriness in half of the hamsters whose gonads had recrudesced spontaneously during 30 weeks of LD 2:22 exposure (16). The times of light onset of the long and short photoperiods between which hamsters were transferred were chosen to ensure rapid entrainment without transient cycles (17). This may have increased the apparent effectiveness of long days in breaking refractoriness (18) and could account for the discrepancies between these findings and Reiter's (19) estimate that at least 14 weeks of long days are required to break testicular refractoriness to short days.

Summary. Melatonin was administered to male hamsters via subcutaneous Silastic or beeswax capsules. Treatments adequate to prevent gonadal regression in LD 2:22 or LD 6:18 also prevented such photoperiods from inducing neuroendocrine refractoriness to the regressive effects of short days. The results are discussed with attention to the mechanisms of melatonin's progonadal effects and the relationship of testicular regression to subsequent gonadal refractoriness.

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