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# Melatonin-Induced Inhibition of Testicular Function in Adult Golden Hamsters[1] (39245)

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The pineal gland has been implicated in the regulation of mammalian reproduction (1, 2). The search for the mechanism(s) by means of which the pineal gland exerts its control over the reproductive system has centered around examining the effects of various pineal hormones, particularly melatonin, on the hypothalamo-hypophyseal-gonadal axis (1-5). Melatonin has been reported to interfere with pituitary gonadotropin release and/or gonadal function in several species (3, 6-9), but the systemic administration of this indole has repeatedly failed to exert antigonadal effects in golden hamsters (2, 10, 11). This lack of effect is paradoxical because pinealectomy has major effects on the reproductive system of the golden hamster and, consequently, this animal has served as the primary model for investigating the interrelationship between photic stimuli, the pineal gland, and the neuroendocrine regulation of reproduction (12, 13).

Preliminary evidence pointing to the possibility that melatonin can provoke testicular atrophy in golden hamsters (14) prompted us to (i) verify the inhibitory effects of melatonin on the testis, and (ii) determine whether or not this indole suppressed photostimulated testicular growth. This report provides unequivocal evidence that melatonin induces testicular regression in sexually mature hamsters and establishes that melatonin can prevent the testicular development that normally occurs when hamsters are transferred from short to long days.

*Materials and methods.* Male golden hamsters *(Mesocricetus auratus)* were reared in our laboratory from animals originally purchased from Lakeview Hamster Colony, Newfield, New Jersey. Wayne Lab-Blox and water were provided *ad libitum.* Hamsters were housed in groups of five to six per cage in a room provided with 14 hr of light and 10 hr of darkness per 24 hr (LD 14:10) prior to experimental treatment. This lighting schedule has been shown to support optimal testicular function in hamsters (15).

Melatonin was purchased from Regis Chemical Co., Morton Grove, Illinois, or Sigma Chemical Co., St. Louis, Missouri. Melatonin from both sources was tested in separate experiments since its biological effects have been shown to vary with the source of the material (16). Silastic capsules (Dow Corning Corp., Medical Products Division, Midland, Michigan; Cat. No. 602-235; 1.47-mm i.d. and 1.96-mm o.d.) containing crystalline melatonin were prepared and placed subcutaneously along the dorsal midline of hamsters anesthetized with sodium pentobarbital. The amount of melatonin released from each capsule *in situ* was determined by weighing the capsules before and at the conclusion of the experiment (14). Melatonin was released from subdermal Silastic capsules at relatively constant rates that were proportional to capsule length (Fig. 1). Importantly, similar release rates were noted with melatonin obtained from either supplier (compare values in Fig. 1 with those reported previously, ref. 14). Moreover, no mortality or evidence of lesions or adhesions was apparent in hamsters receiving melatonin-filled capsules.

In one study, male hamsters (12 weeks of age) were implanted with empty Silastic capsules or melatonin-filled Silastic capsules

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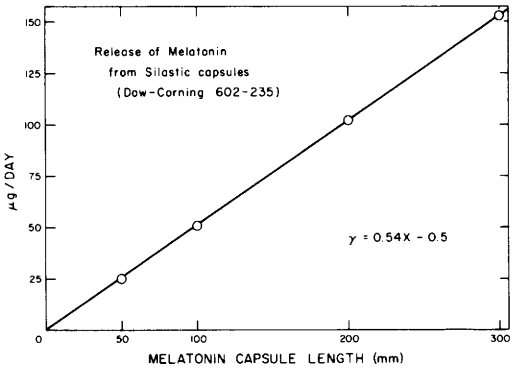


FIG. 1. Relationship between the length (mm) of melatonin-filled Silastic capsules and the amount of melatonin ($\mu$g/day) released *in situ*. Release rates were calculated by determining the loss in capsule weight 60 days after subcutaneous implantation in hamsters. Each value represents the mean ± standard error (within the point symbol) of five to six hamsters.

that were 50, 100, 200, or 300-mm long, respectively. A set of experiments was performed with melatonin purchased from Regis Chemical Co. and Sigma Chemical Co. Hamsters were exposed to an LD 14:10 light cycle prior to and during the experiment. The average weight of the testes of six hamsters autopsied at the time of capsule implantation was 3087 ± 88 mg. Hamsters were killed 60–80 days after the capsules were implanted to assess the response of the testes to this mode of melatonin administration.

In another study, sexually mature hamsters (12 weeks of age) were transferred from a LD 14:10 photostimulatory light cycle to a nonstimulatory photoperiod (6 hr light/24 hr; LD 6:18). The weight of testes from six hamsters averaged 3250 ± 116 mg at the time the animals were transferred to the LD 6:18 light cycle. After exposure to the LD 6:18 light cycle for 60 days, a subsample of nine hamsters was autopsied (mean paired testis weight = 263 ± 19 mg), and the remaining animals were implanted with Silastic capsules. Three groups of animals (five to six per group per cage) remained on the LD 6:18 photoperiod and received either an empty Silastic capsule or a 25- or 200-mm melatonin-filled Silastic capsule. An additional five groups of animals (five to six per group per cage) were transferred to a photostimulatory LD 14:10 light cycle at the time they received either an empty Silastic capsule or a 25-, 50-, 100-, or 200-mm melatonin-filled Silastic capsule. The melatonin used in this study was purchased from the Sigma Chemical Co. All hamsters were killed 42 days after capsule insertion. Paired testis weight was determined, and one of the testes was fixed and used to determine the relative number of germ cells at stage VII of the cycle of the seminiferous epithelium (17). Germ cell nuclei were counted in 25 "round" tubular cross sections, and all nuclear counts were corrected for differences in nuclear diameter by Abercrombie's formula (18) and for tubular shrinkage by a Sertoli cell correction factor (19).

*Results.* The average weight of the testes of control animals, maintained under the photostimulatory LD 14:10 photoperiod and treated with empty Silastic capsules, was similar to that of untreated hamsters autopsied at the time the experiment was initiated (Fig. 2). In contrast, partial or complete testicular regression was observed in

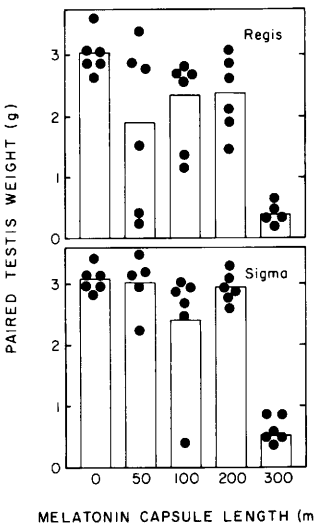


FIG. 2. Average testis weight of adult hamsters exposed to an LD 14:10 light cycle and implanted with melatonin-filled Silastic capsules of various sizes for 60–80 days. Control hamsters received an empty 50-mm Silastic capsule. Paired testis weight of individual hamsters is represented by the closed circles. Results depicted in the upper panel were obtained with melatonin purchased from Regis Chemical Co., while those in the bottom panel were obtained with melatonin obtained from the Sigma Chemical Co.

several hamsters that received 50-, 100-, and 200-mm melatonin-filled Silastic capsules. Marked testicular atrophy was apparent in all hamsters implanted with 300-mm melatonin-filled Silastic capsules (Fig. 2). Similar antigonadal responses were found with melatonin obtained from either commercial source. No differences ($P > 0.50$) in body weight were apparent between control and melatonin-treated hamsters.

Marked testicular regression occurred in the hamsters exposed to 60 days of LD 6:18 as evidenced by the tenfold decline in paired testis weight and the near absence of step 7 spermatids at stage VII of the cycle of the seminiferous epithelium (Fig. 3 and Table I). This degree of testicular atrophy was expected since exposure to nonstimulatory photoperiods leads to a reduction in pituitary gonadotropin release and to an eventual reduction in testicular weight and steroidogenic activity in golden hamsters (17, 20). Hamsters treated with empty Silastic capsules and exposed to short days, for an additional 42 days, exhibited a slight (33%) increase in testicular weight (Fig. 3). This increase was anticipated and has previously been shown to be associated with the onset of spontaneous testicular growth that occurs in hamsters maintained on nonstimulatory photoperiods for extended periods (12, 21). Administration of melatonin, via 25- or 200-mm Silastic capsules, had no measurable effect on the average weight of the testes or the spermatogenic activity of hamsters exposed to short days (Fig. 3, Table I). In contrast, photoinduced testicular growth, normally seen when hamsters are transferred from nonstimulatory to stimulatory photoperiods (17, 21), was suppresed in all hamsters that were transferred to LD 14:10 and received melatonin-filled Silastic capsules ranging between 25 to 200 mm (Fig. 3). The relative number of spermatocytes and spermatids, present at stage VII of the cycle of the seminiferous epithelium, were markedly suppressed in melatonin-treated hamsters exposed to long days in comparison to control hamsters receiving empty capsules (Table I). The relative number of germ cells present in the testes of control hamsters receiving empty capsules and exposed to LD 14:10 for 42 days was nearly comparable to that seen in hamsters maintained on long days for 12 weeks (Table I).

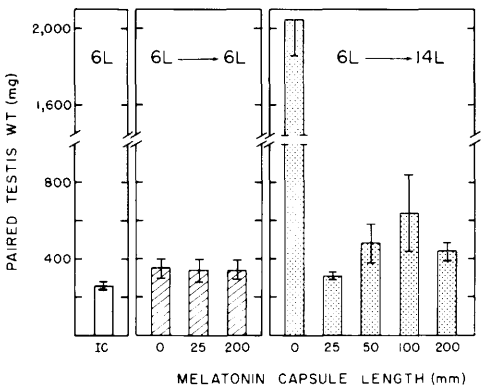


FIG. 3. Average testis weight of hamsters implanted with either an empty Silastic capsule, or melatonin-filled Silastic capsules of various sizes. Prior to capsule implantation all hamsters were exposed to short days (LD 6:18) for 60 days to induce testicular atrophy. The paired testis weight of control animals sacrificed at this time is presented in the left panel of the figure. After capsule implantation, some of the animals were maintained on an LD 6:18 photoperiod for an additional 42 days (middle panel) while others were transferred to an LD 14:10 photoperiod for 42 days (right panel).

*Discussion.* The results demonstrate, for the first time, that the systemic administration of melatonin via subdermal Silastic capsules can suppress testicular growth that normally occurs when adult golden hamsters are transferred from nonphotostimulatory to photostimulatory day lengths. In contrast to these findings, melatonin failed to affect light-induced testicular development in golden hamsters when administered via subcutaneous pellets mixed with beeswax (10). However, melatonin has been shown to inhibit photoinduced testicular growth in weasels (6) and Djungarian hamsters (9) when administered via beeswax pellets. It seems likely that we were able to demonstrate a pronounced antigonadal effect of melatonin in photostimulated golden hamsters because this indole was released from the subdermal Silastic capsules at relatively constant rates over prolonged periods.

Previously we reported that Silastic capsules releasing from 25–100 $\mu$g of melatonin per day caused marked testicular atrophy in sexually mature hamsters maintained on a LD 14:10 light cycle (14). However, in the present study, capsules releasing 25–100 $\mu$g of melatonin/day (50- to 200-mm capsule length) did not alter testicular weight in

TABLE I. RELATIVE NUMBER OF GERM CELL NUCLEI IN TUBULAR CROSS-SECTIONS AT STAGE VII OF THE CYCLE OF THE SEMINIFEROUS EPITHELIUM.[a]

| Melatonin-filled capsule length (mm) and duration of treatment (days) | LD cycle (hr) and duration (days) | Relative number of germ cells | | | |
|---|---|---|---|---|---|
| | | Type A spermatogonia | Preleptotene spermatocytes | Pachytene spermatocytes | Step 7 spermatids |
| None | 14:10 (84) | 1.06 ± 0.22 | 26.7 ± 1.3 | 26.0 ± 1.0 | 99.7 ± 3.1 |
| None | 6:18 (60) | 0.81 ± 0.17 | 10.3 ± 0.7 | 6.8 ± 0.6 | 2.9 ± 0.4 |
| None | 6:18 (102) | 0.86 ± 0.11 | 11.3 ± 0.5 | 8.1 ± 0.2 | 4.4 ± 0.4 |
| 200 (42) | 6:18 (102) | 0.83 ± 0.14 | 8.4 ± 0.8 | 6.2 ± 0.4 | 2.1 ± 0.1 |
| None | 14:10 (42) | 0.96 ± 0.12 | 27.8 ± 2.2 | 26.9 ± 2.1 | 81.0 ± 2.9 |
| 25 (42) | 14:10 (42) | 0.90 ± 0.14 | 9.1 ± 1.0 | 3.7 ± 0.6 | 1.1 ± 0.7 |
| 200 (42) | 14:10 (42) | 0.88 ± 0.09 | 7.2 ± 0.7 | 4.1 ± 0.2 | 0.2 ± 0.1 |

[a] Each value represents the mean ± SE for a testis from five hamsters.

most of the treated animals, while capsules releasing about 150 μg/day (300-mm long) induced marked testicular atrophy in all hamsters maintained on long days. This result confirms our preliminary finding that melatonin can induce testicular atrophy in sexually mature hamsters but underlines the fact that the amount of melatonin required to evoke antigonadal responses may vary between experiments.

The possibility that differences in commercially supplied melatonin might account for the variation in the amount of material required to induce antigonadal responses, prompted us to compare the effects of melatonin purchased from two different suppliers. Melatonin used in our preliminary study was obtained from Regis Chemical Co. (14), while that used in the present study was purchased from Regis as well as Sigma Chemical Co. However, the material from both suppliers produced equivalent antigonadal responses in the present study (Fig. 2).

Differences in the amount of melatonin required to induce antigonadal responses in adult hamsters maintained on long days may be related to the time of year at which the experiments are performed. This possibility must be entertained since our initial experiments were performed between November and December (14), whereas the present work was performed in May–July. Uncontrolled seasonal changes may have occurred in our light-controlled animal rooms that altered the amount of melatonin required to suppress testicular function in adult golden hamsters.

It is not clear whether the antigonadal effects of melatonin observed in the present study are due to a direct or indirect action of melatonin on the neuroendocrine–testicular axis. While the mechanism(s) by which melatonin influences the reproductive system is unknown, it is now quite clear that the systemic administration of this indole provokes profound differential changes in testicular function in golden hamsters. Melatonin prevents the testicular regression that normally occurs in light-deprived hamsters (10, 14). On the other hand, melatonin exerts antigonadal effects in sexually mature hamsters and suppresses light-induced testicular recrudescence (14; Figs. 2 and 3). The effects of melatonin depend on dosage, method of administration, and the photoperiodic conditions to which male hamsters have been exposed.

*Summary.* Melatonin (12–100 μg/day) administered via subcutaneous Silastic implants prevented or suppressed light-induced testicular recrudescence in adult golden hamsters. In addition, melatonin (150 μg/day) induced marked testicular regression in sexually mature hamsters maintained on photostimulatory long days. These results clearly establish that exogenous melatonin can inhibit gonadal function in adult male hamsters.

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