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Antigonadal Effect of Melatonin in Pinealectomized and Intact Male Hamsters[1] (39738)

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The pineal gland plays a major role in the regulation of the hypothalamic-hypophyseal-gonadal axis in many mammals (1). Pinealectomy, as well as the administration of pineal extracts, has been shown to alter the neuroendocrine-gonadal system in a number of animals (2-7). Numerous investigators have attempted to determine the pineal factor(s) responsible for the effect of the pineal gland on the reproductive system. Two general classes of antigonadal compounds (indoles and polypeptides) have been isolated from the pineal gland (4-6, 8), and much of the search for a pineal antigonadal factor has centered around the indole *N*-acetyl-5-methoxytryptamine, commonly known as melatonin.

Recent studies have shown that melatonin can induce testicular atrophy in golden hamsters maintained on stimulatory photoperiods (i.e., $\geq$12.5 hr of light/24 hr) and can inhibit light-induced testicular development in hamsters transferred from nonstimulatory (i.e., <12 hr of light/24 hr) to stimulatory photoperiods (9, 10). In addition, pronounced antigonadal effects of melatonin have been observed in other photoperiodic mammalian species (11-13). It is not known whether exogenous melatonin is acting directly or indirectly on the neuroendocrine-gonadal axis to inhibit gonadal activity. It has been suggested that melatonin may alter the neuroendocrine-gonadal system by affecting the synthesis and/or release of other substances (e.g., polypeptides) by the pineal gland (4, 14). The objective of the present study was to determine whether exogenous melatonin can inhibit gonadal activity independently of the pineal gland.

Materials and methods. Eight-week-old male golden hamsters (*Mesocricetus auratus*) were purchased from Lakeview Hamster Colony, Newfield, N. J. Animals were housed in groups of four to five per cage in an LD 14:10 (14 hr of light/24 hr) room until experiments were initiated. This lighting schedule has been shown to maintain optimal testicular function in hamsters (e.g., paired testis weight, 2800-3000 mg) (15).

Melatonin was purchased from Sigma Chemical Co., St. Louis, Mo. Silastic capsules (Dow Corning, catalog no. 602-235) containing crystalline melatonin were prepared as previously described (9) and placed subcutaneously along the dorsal midline of hamsters anesthetized with sodium pentobarbital. Melatonin is released from subdermal Silastic capsules at relatively constant rates that are proportional to capsule length and the release rate is about 50 μg/day/100 mm of capsule length (9, 10). Multiple numbers of 50-mm capsules were used to achieve values greater than 50 mm. Each 50-mm capsule contains approximately 65 mg of crystalline melatonin.

Pinealectomy was performed under sodium pentobarbital anesthesia following the procedure of Hoffman and Reiter (2). A small piece of bone overlying the confluence of sinuses is removed and the pineal gland with the attached pineal stalk is plucked via fine forceps from the area between the occipital poles of the cerebral hemispheres and the tectum of the midbrain. During sham pinealectomy a piece of the skull cap is removed, but the pineal gland is left intact.

In the first experiment 12-week-old sexually mature male hamsters were transferred from a photostimulatory (LD 14:10) to a nonstimulatory (LD 6:18) light cycle that is known to induce complete testicular atrophy in about 9 weeks (16). After a 65-day exposure to LD 6:18, six initial control animals (IC) were sacrificed and paired testis weight was determined. The remaining

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animals were either sham-pinealectomized (intact) or pinealectomized (Px). At the time of surgery the animals were implanted with empty Silastic capsules or melatonin-filled Silastic capsules that were 25-, 100-, or 300-mm long (seven to nine animals per group). All of the animals were then moved back to the photostimulatory LD 14:10 light cycle and were sacrificed 45 days later to determine the response of the testis to this mode of melatonin administration.

In a second study 10-week-old male hamsters that had been maintained on LD 14:10 were either sham-pinealectomized (intact) or pinealectomized (Px). The animals were implanted with melatonin-filled Silastic capsules that were 300-mm long and left on LD 14:10. At the time of surgery five animals were sacrificed as initial controls and paired testis weight was determined. Eighty days later a subsample of five intact animals was autopsied. In contrast to an earlier study (10) in which melatonin-filled capsules that were 300-mm long induced testicular atrophy in all intact animals, only two of the five intact animals in the present study showed evidence of testicular regression (paired testis weight, 1705 and 848 mg). Therefore, the remaining animals were implanted with an additional 300 mm of melatonin-filled capsules. Sixty days later the intact ($N = 5$) and pinealectomized ($N = 5$) hamsters were sacrificed.

Analysis of variance was used to determine the significance of treatment effects, and differences between treatments were tested for significance using the Student Newman–Keuls sequential range test (17).

Results. The exposure to the nonstimulatory LD 6:18 light cycle resulted in complete testicular regression while the transfer of intact hamsters from LD 6:18 to the stimulatory LD 14:10 photoperiod resulted in the expected increase in testicular weight (Fig. 1) (16, 18). Testicular growth occurred to the same degree in intact and pinealectomized hamsters receiving empty Silastic implants as a result of exposure to the LD 14:10 light cycle. Photic-induced testicular recrudescence was prevented or suppressed in both intact and pinealectomized animals implanted with either 25-, 100-, or 300-mm long melatonin-filled Silastic

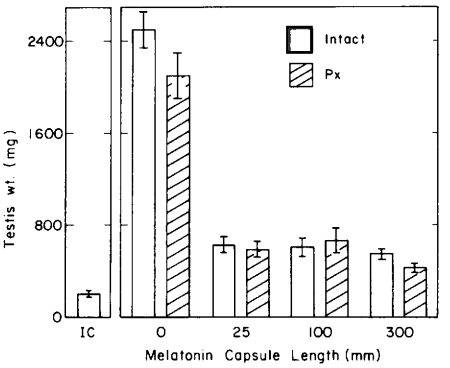

FIG. 1. Mean paired testis weight (± SE) of intact and pinealectomized (Px) hamsters that were implanted with either an empty Silastic capsule (0) or melatonin-filled Silastic capsules of various sizes. Prior to implantation of the capsules, the animals were maintained on an LD 6:18 photoperiod for 65 days to induce testicular atrophy. The testis weight of initial control (IC) animals sacrificed at this time is shown in the left panel. After capsule implantation, all of the animals were transferred to an LD 14:10 photoperiod and sacrificed 45 days later.

capsules. All of the groups of animals treated with melatonin had significantly smaller testes ($P < 0.01$) than the intact and pinealectomized hamsters receiving empty capsules, while no significant difference in testicular weight was observed between any of the melatonin-treated groups. Although testicular growth was inhibited in all of the melatonin-treated animals, some testicular growth did occur during the time the animals were being administered melatonin (Fig. 1; compare initial control animals with melatonin-treated animals).

In the second study melatonin provoked testicular atrophy in sexually mature intact and pinealectomized hamsters maintained under a stimulatory LD 14:10 light cycle (Fig. 2). At the start of the experiment testis size was at, or near, a maximum as revealed by autopsy of a subsample of five initial control animals. After an 80-day exposure to melatonin-filled capsules that were 300-mm long followed by a 60-day exposure to melatonin-filled capsules that were 600-mm long, pronounced testicular regression had occurred in both pinealectomized and intact animals. This regression occurred in spite of the fact that the animals were maintained on a photostimulatory LD 14:10 light cycle

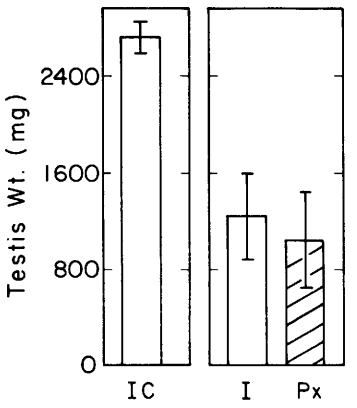

FIG. 2. Mean paired testis weight (± SE) of intact (I) and pinealectomized (Px) hamsters that were implanted with 300 mm of melatonin-filled capsules for 80 days followed by 600 mm of melatonin-filled capsules for 60 days. The animals were maintained on a photostimulatory LD 14:10 light cycle throughout the study. The testis weight of initial control (IC) animals sacrificed at the time of capsule implantation is shown in the left panel. Both pinealectomized and intact animals treated with melatonin had significantly ($P < 0.01$) smaller testes than the initial control animals.

throughout the course of the study. Reiter (19) has shown that the testes of both intact and pinealectomized hamsters remain enlarged (weight ≈3000 mg) for at least 60 weeks when the animals are maintained continuously on LD 14:10. Therefore, the decrease in testicular size (55% in intact and 62% in pinealectomized animals) is apparently due to the melatonin treatment.

Discussion. Previous studies have shown that melatonin can induce testicular atrophy in sexually mature hamsters maintained on stimulatory photoperiods and inhibit light-induced testicular development in hamsters transferred from nonstimulatory to stimulatory photoperiods (9, 10). The present studies confirm these findings and demonstrate that the antigonadal action of exogenous melatonin can occur independently of the pineal gland. Thus, under the present experimental conditions, the antigonadal action of melatonin is not due to an alteration of the synthesis of pineal peptides or any other compounds secreted by the pineal gland.

Melatonin has also been found to act in a progonadal fashion by preventing gonadal regression in hamsters transferred from photostimulatory to nonstimulatory photoperiods (9, 20, 21). Whether or not this progonadal effect of melatonin is also independent of the animal's own pineal gland is not known. Analogous experiments to those reported here cannot be performed to answer this question since the removal of the pineal gland by itself will prevent light-induced gonadal regression in hamsters (19).

While melatonin prevented the dramatic increase in testicular size observed when hamsters are transferred from short to long days, it is evident that some testicular growth was occurring during the time of melatonin treatment (Fig. 1). When male hamsters are held on short photoperiods, the initial collapse of the gonads is eventually followed by "spontaneous recrudescence" of the testes after a 20- to 25-week exposure to the nonstimulatory light cycle (16, 19). The small amount of testicular growth in the melatonin-treated animals in the present study may be due to the inability of melatonin to totally suppress photic-induced gonadal growth or, alternatively, melatonin may not be able to prevent spontaneous testicular recrudescence. It appears that in the djungarian hamster (*Phodopus sungorus*) melatonin does not prevent spontaneous testicular development but does prevent photic-induced gonadal development (12).

Throughout this and previous papers we have referred to the inhibitory effect of melatonin on the reproductive system as "antigonadal." This term has been employed because the activity of the testis has been used as an endpoint to ascertain the effects of melatonin on reproductive function. This term is not meant to imply that the observed effects are necessarily due to a direct action of melatonin on the gonads. While there is some evidence that melatonin can have a direct effect on gonadal activity (22–25), other lines of evidence suggest that melatonin alters gonadal activity by altering hypothalamic–hypophyseal activity (26–30). By whatever means melatonin is acting, it is clear from the present studies that the inhibitory effects of exogenous melatonin are not dependent upon pineal activity.

Summary. The administration of melatonin (12–150 μg/day) via subcutaneous Silastic capsules inhibited photic-induced tes-

ticular recrudescence to the same degree in both pinealectomized and intact hamsters. Melatonin (300 μg/day) also induced marked testicular regression in sexually mature intact and pinealectomized hamsters maintained on a photostimulatory LD 14:10 light cycle. The similar responses of pinealectomized and intact hamsters to melatonin treatment indicate that the effects of exogenous melatonin are not mediated via the pineal gland.

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