PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 149, 290–293 (1975)

Melatonin-Pineal Relationships in Female Golden Hamsters[1] (38791)

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(Introduced by Edward G. Rennels)

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In the rat, melatonin has frequently been designated as the pineal hormone which inhibits normal pituitary-gonadal interactions (1, 2). In the golden hamster, however, this indole even when administered daily for prolonged periods (8 wk) is capable of depressing growth of the gonads (3, 4). This has led to the speculation that melatonin may not be the pineal antigonadotrophic principle in hamsters. The present paper reports that melatonin administered subcutaneously in beeswax to female hamsters actually prevents the pineal gland from suppressing the neuroendocrine-reproductive axis and sheds further doubt on its antigonadotrophic capabilities in this species.

Materials and Methods. Thirty-eight adult (65–70 g) female hamsters (LVK:LAK strain, Lakeview Hamster Colony, Newfield, N.J.) were divided into four groups. Three of the groups were blinded by bilateral orbital enucleation. One of these groups was pinealectomized while another received weekly subcutaneous implants of a melatonin:beeswax pellet (1 mg melatonin in 24 mg beeswax). Before the surgical procedures the animals received sodium pentobarbital while during the pellet implanations the hamsters were anesthetized with ether. The surgical procedures and the pellet preparations were similar to those described in an earlier study (5). Hamsters were housed 3 or 4 per cage in a light (LD 14:10) and temperature (22 ± 1°) controlled room. As food, Wayne Lab Blox was provided *ad libitum*.

After 8 wk of treatment all animals were killed (between 0800–1000 hr) by rapid decapitation and trunk blood was collected in heparinized tubes. Plasma samples were stored frozen until hormone assays. Body, ovarian, uterine and anterior pituitary weights were recorded and pituitary glands were retained for hormone analyses.

Immunoreactive plasma and pituitary luteinizing hormone (LH) and prolactin were determined by radioimmunoassays described by Goldman and Porter (6) for hamster LH and by Donofrio *et al.* (7) for hamster prolactin. Reagents for the assays were kindly provided by Rat Hormone Distribution Program, NIAMD. Because of lack of parallelism between rat and hamster prolactin inhibition curves, hamster prolactin data are expressed in terms of a pool of standard hamster anterior pituitaries (SHAP) (7). Data were statistically analyzed in a Programma 101 Computer using an analysis of variance and the Students' *t* test.

Results. After 8 wk of treatment the mean body weights of all groups of hamsters were similar (Table I). Ovaries of blinded hamsters were significantly enlarged; the ovarian enlargement in the light deprived hamsters was prevented by pinealectomy or by melatonin treatment. Uteri of blinded hamsters were markedly atrophic, a response that was again obviated in pinealectomized or melatonin treated hamsters. Anterior pituitary glands of blinded hamsters were depressed in weight compared to those of intact control hamsters while animals that received weekly implants of melatonin had heavier than normal anterior pituitary glands.

The concentration of LH in the pituitary glands of blinded hamsters was elevated over that of intact control hamsters (Fig. 1, top); however, blinded hamsters that were either pinealectomized or melatonin treated had pituitary LH values similar to

[1] Supported by NSF Grant No. GB-43233X.

[2] U.S.P.H.S. Career Development Awardee, HD-42398.

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TABLE I. INFLUENCE OF PINEALECTOMY AND MELATONIN TREATMENT ON BODY WEIGHTS AND ABSOLUTE (mg) AND RELATIVE (mg/100 g BW; PARENTHESIS) ORGAN WEIGHTS OF ADULT FEMALE GOLDEN HAMSTERS. ANIMALS WERE KEPT IN 14 HR LIGHT PER DAY.

Treatment	N	Body wt (g)	Ovaries	Uterus	Ant. pit.
Intact	10	154 ± 7	30.4 ± 3.1	354 ± 27	4.18 ± 0.24
			(19.7 ± 1.7)	(230 ± 22)	(2.71 ± 0.16)
Blind	10	162 ± 6	45.8 ± 2.9[b]	153 ± 19[c]	3.24 ± 0.09[b]
			(28.2 ± 2.1[b])	(94 ± 15[c])	(2.00 ± 0.05[b])
Blind; pinealectomized	9	148 ± 6	27.4 ± 2.5	331 ± 22	3.81 ± 0.16
			(18.5 ± 1.1)	(224 ± 17)	(2.57 ± 0.11)
Blind; melatonin	9	151 ± 4	28.1 ± 3.3	369 ± 36	4.53 ± 0.19[a]
			(18.6 ± 1.9)	(244 ± 28)	(3.00 ± 0.15)

[a] $P < 0.05$, [b] $P < 0.02$ and [c] $P < 0.001$ vs. all other groups.

those of the control hamsters. Pituitary prolactin levels were significantly depressed after blinding alone (Fig. 1, bottom); pituitary prolactin levels were partially restored by pinealectomy and completely restored after melatonin treatment.

Compared to plasma titers in intact control hamsters, none of the experimental treatments appreciably influenced the circulating levels of LH (Fig. 2). Despite the remarkable drop in pituitary prolactin stores in the light deprived hamsters, plasma levels of the hormone were not altered (Fig. 2). Hamsters that received weekly subcutaneous implants of melatonin had higher than normal circulating prolactin titers.

Discussion. The present findings confirm the observations (8) that light deprivation is accompanied by functional regression of the peripheral sexual organs and elevation of pituitary LH with depression of hypophyseal prolactin stores. That the changes are almost completely reversed by pinealectomy is also well known (5). Contrary to observations in the rat, when the ovaries of the hamster functionally involute they actually increase in weight due to a pronounced proliferation of the interstitial elements; this is accompanied by repression of follicular development and by the nearly complete absence of corpora lutea. Hormones determining structural growth of the hamster ovary have been studied in detail by Greenwald (9). The major new finding in the present report is that melatonin acts like pinealectomy in preventing gonadal atrophy in light deprived female hamsters.

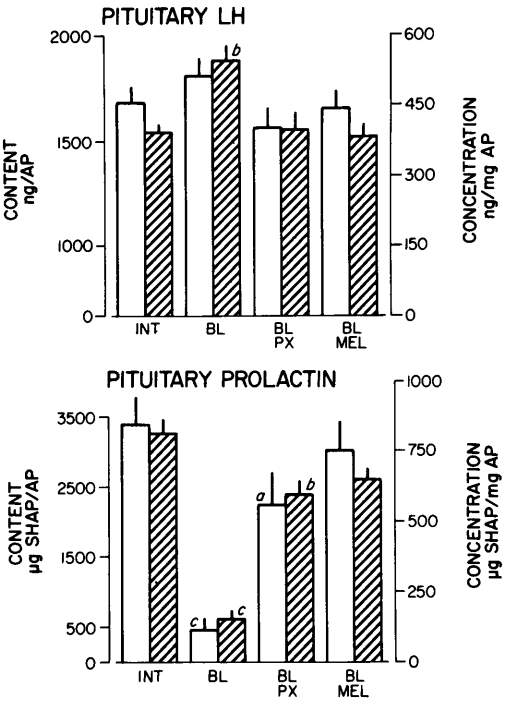

FIG. 1. Pituitary LH and prolactin levels in adult female golden hamsters. Treatment period was 8 wk. INT, intact control hamsters; BL, blinded; PX, pinealectomized; MEL, weekly implantation of a melatonin:beeswax pellet. [a] $P < 0.05$; [b] $P < 0.02$ and [c] $P < 0.001$ vs. INT.

This is the first report that melatonin actually prevents the pineal gland from interfering with normal pituitary-ovarian relationships in female hamsters. Melatonin has frequently been proposed as the pineal hormone; this judgment was usually made

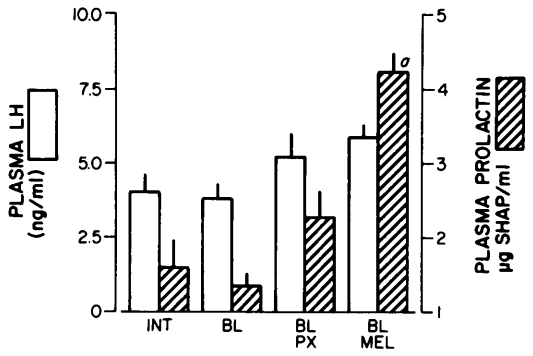

FIG. 2. Plasma LH and prolactin levels in adult female golden hamsters. Treatment period was 8 wk. INT, intact control hamsters; BL, blinded; PX, pinealectomized; MEL, weekly implants of a melatonin:beeswax pellet. $^{a}P < 0.01$ vs. all other groups.

in reference to data obtained using the rat (1, 2). In hamsters exposed to long daily photoperiods, however, melatonin has never been shown to inhibit reproduction (3, 4). These findings coupled with the observations reported herein, raise doubt about the antigonadotrophic role of melatonin in female golden hamsters.

The mechanisms whereby melatonin negated the inhibitory influence of the pineal gland on the neuroendocrine-reproductive axis cannot be determined from the present study. Possibly melatonin's site of action is within the pineal gland where it somehow influences the metabolism of the real antigonadotrophic factor. The indole may have inhibited either the formation or the discharge of the hypothetical inhibitory substance; this inhibitory factor could well be a polypeptide (10, 11). Although he did not specify the significance of such an action, Quay (12) has contended for several years that pineal melatonin may act intrinsically.

Another explanation as to how melatonin stymied the action of the pineal gland in blinded female hamsters includes the stimulation of release of a progonadotrophic substance. There is little experimental evidence to support the idea of a gonad-stimulating factor from the pineal gland. Melatonin may also have altered the sensitivity of the hypothalamo-pituitary system and thereby rendered the pineal antigonadotrophic factor incapable of depressing reproductive physiology. Finally, melatonin may have had a direct trophic influence on the ovaries and uterus. Regardless of how the indole achieved its result, it appears that melatonin is probably not the pineal antigonadotrophic substance in the female golden hamster. In two species of hamsters, melatonin appears incapable of inhibiting testicular function as well (5, 13).

Melatonin has been shown to stimulate the release of prolactin from the rat pituitary. Kamberi *et al.* (14) found that when melatonin was injected into the ventricular system of the brain, circulating prolactin titers immediately rose. In the present study, hamsters which had melatonin:beeswax pellets subcutaneously also had elevated plasma levels of prolactin (Fig. 2). Melatonin's action on prolactin may be mediated by an inhibition of prolactin release inhibiting factor (14).

Summary. Light deprivation by blinding in female hamsters was followed by a regression of the reproductive organs, an elevation of pituitary LH concentration and a depression of pituitary prolactin levels. Pinealectomy negated almost completely the effects of light deprivation on the neuroendocrine-reproductive axis. Weekly subcutaneous implants of a melatonin:beeswax pellet completely prevented the pineal gland from inhibiting reproductive physiology in blinded hamsters. The findings suggest that melatonin is not the pineal antigonadotrophic factor in female golden hamsters. Melatonin implanted hamsters also had higher than normal levels of plasma prolactin.

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Received December 16, 1974. P.S.E.B.M. 1975, Vol. 149.