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EFFECTS OF PHOTOPERIOD AND MELATONIN ON REPRODUCTION IN THE SYRIAN HAMSTER

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SUMMARY

The Syrian hamster is a spontaneous ovulator and, in the wild, a seasonal breeder. Daylength (*i.e.* photoperiod) appears to be an important factor in the regulation of seasonal periodicity in this species. Female hamsters became acyclic after 6-8 weeks exposure to « short days » (*i.e.* a 10-hour photoperiod). During the period of acyclicity these animals displayed a pronounced diurnal rhythmicity in LH secretion with peak serum LH concentrations during the mid-afternoon. Serum FSH and progesterone concentrations, showed a similar rhythmicity to that observed for LH, but the fluctuations were of lesser magnitude. Ovariectomy reduced serum progesterone levels and abolished the afternoon rise in serum progesterone. Serum LH was unaffected by ovariectomy while serum FSH levels were somewhat increased. Following removal of the ovaries, both gonadotropins continued to display peak concentrations in the serum during the afternoon.

Daily injections of melatonin, a pineal product, inhibited ovulatory cyclicity in hamsters maintained on « long days » (*i.e.* a 14-hour photoperiod) when the compound was administered 4 hours prior to lights off (15.00 h). This effect was not present when the melatonin was given at 08.00 h nor was it present in pinealectomized hamsters. Similar inhibitory effects of melatonin on the male hamster reproductive system have been observed, and these effects are also dependent upon the time of day at which the compound is administered.

INTRODUCTION

The Syrian hamster appears to utilize daylength as a primary environmental cue for regulating seasonal reproductive cycles. In the laboratory, hamsters show regression of the gonads and sex accessories following exposure to short daily photoperiods (REITER, 1974). Gonadal regression does not occur if the animals are pinealectomized or if they are superior cervical ganglionectomized prior to light-deprivation.

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(The superior cervical ganglion provides the sole source of innervation for the mammalian pineal.) Therefore, it has been proposed that the pineal gland produces a hormone which is « anti-gonadal » in nature and that the secretion of this hormone is responsible for mediating the effects of photoperiod upon the reproductive system (REITER, 1968).

At least three possible sites of action for a pineal anti-gonadal hormone might be proposed. First a direct effect on the gonads is possible. However, this appears unlikely since REITER (1967) observed that blinded hamsters still showed gonadal responses to injections of gonadotropic hormones (FSH and LH). Nevertheless, the possibility of a partial reduction in sensitivity to gonadotropins has not been conclusively ruled out. The other two possible sites of pineal hormone action that have been suggested involve the regulation of gonadal activity through control of pituitary gonadotropin secretion. This might occur by way of either a direct effect on the anterior pituitary or on brain areas controlling the anterior pituitary. Such indirect anti-gonadal control by the pineal hormone, presumably working via interference with the synthesis and/or release of gonadotropins, is the mechanism favored by most investigators. Evidence for an effect of shortened photoperiods on the pattern of gonadotropin secretion has been reported recently for male (BERNDSTON and DESJARDINS, 1974) and female (SEEGAL and GOLDMAN, 1975) hamsters.

The present report describes experiments designed to further investigate the effects of photoperiod on the secretion of gonadotropins in the female hamster and also to study the mechanisms by which photoperiodic changes are translated into alterations in gonadotropin secretion. The findings which are reported here suggest that, at least in the female, the light-induced acyclic state is accompanied by a marked change in the *pattern* of gonadotropin secretion. The results further indicate a possible diurnal rhythm in sensitivity to the « antigonadal » effects of melatonin in both male and female hamsters.

MATERIALS AND METHODS

Animals

A group of 15 adult female hamsters obtained from Lakeview Hamster Colony (Newfield, New Jersey) were housed in a « short day » environment in which they received 10 hours of illumination daily (05.00-15.00). A second group of hamsters (males and females) obtained from our own colony (which is derived from Lakeview stock) remained housed in a room in which the lights were on for 14 hours each day (05.00-19.00). These animals were used to study the effects of exogenous melatonin on reproductive capacity. In a final experiment male hamsters obtained from our colony were housed on a 13-hour photoperiod (lights on 05.00-18.00) and treated with melatonin. All animals received food (Wayne Lab Blox) and water *ad libitum*.

Gonadotropins and progesterone in hamsters on « short days »

The females received from Lakeview Hamster Colony were checked daily for estrous vaginal discharge beginning 4 weeks after being placed in the short day environment. After 6-8 weeks on short days each of the 15 females had become acyclic, defined as the lack of an estrous smear (ORSINI, 1961) for more than 8 consecutive days. All hamsters were checked daily for the remainder of the experiment to ensure that each female was still acyclic. Two to four weeks after the cessation of cyclicity each animal was bled by heart puncture under light ether anesthesia at

each of three times : 10.00, 15.00, and 19.00. These bleedings were made over a three day period such that each animal was bled once each day. Approximately 0.7-1.0 ml of blood was collected at each time, centrifuged at 4000 RPM for 20 minutes, and the sera were frozen at —20°C until assayed for hormone content. Two weeks following the initial bleedings 13 of these acyclic hamsters (two animals died following the first bleeding) were assigned to one of two treatment groups. One group of 7 hamsters was ovariectomized (ovx) under ether anesthesia, while the remaining 6 animals were sham-ovx. Three to four days following surgery each female was bled at 10.00 h and 15.00 h. The blood was centrifuged and stored as previously described.

Melatonin treatment

Melatonin (Schwartz Mann) was administered subcutaneously in 0.1 ml sesame oil. In the first experiment injections were given daily at either 08.00 h or at 15.00 h to adult hamsters of both sexes. Some of the animals were pinealectomized prior to beginning treatment with melatonin. Pinealectomy was performed by a slight modification of a method described by HOFFMAN and REITER (1965). The females were checked for vaginal estrous cycles as described above. The males were castrated after 7 weeks of treatment and the testes were weighed. In a follow-up experiment adult male hamsters were housed in a 13-hour photoperiod and injected with melatonin at 09.00 h or 15.00 h. In this study each animal received two injections daily, with oil only being administered at one time and melatonin at the other time. A control group received oil at both times.

Hormone assays

Sera were assayed for LH, FSH, and progesterone. Serum levels of luteinizing hormone (LH) were determined by radioimmunoassay carried out with antiserum raised against ovine LH (NISWENDER *et al.*, 1968). Follicle-stimulating hormone (FSH) assays employed the NIAMDD Rat FSH Kit with Anti-Rat FSH S-5 or S-6. LH values are expressed in terms of NIAMDD Rat LH-RP-1. FSH is expressed in terms of NIAMDD Rat FSH-RP-1. These assay systems have been validated for use in the hamster (BAST and GREENWALD, 1974 ; BLAKE *et al.*, 1973). Serum progesterone was also assayed by radioimmunoassay following the procedure described by ABRAHAM *et al.* (1971) with modifications in the method of extraction (JOHANSSON, 1970) and using a highly specific antibody to progesterone (NISWENDER, 1973). The sensitivities of these assays were the following : LH approximately 5 ng, FSH approximately 15 ng, and progesterone about 25 pg. Results were analyzed using analysis of variance (ANOVA) and the sign test.

RESULTS

Photoperiod-induced acyclic female hamsters

LH : An overall analysis of LH values showed no overlap in serum values between the 15.00 h levels and either the 10.00 h or 19.00 h levels. Serum LH rose at least 10-fold between 10.00 h and 15.00 h and then fell to « baseline » at 19.00 h (fig. 1). Ovariectomy (ovx) had no effect upon serum LH (fig. 2). In both the sham-ovx and ovx hamsters LH levels were less than 80 ng/ml at 10.00 h and increased to 436 and 427 ng/ml in sham-ovx and ovx animals, respectively at 15.00 h.

FSH : An overall analysis of variance indicated a significant difference in FSH levels throughout the day in the acyclic hamsters ($P < .001$). The FSH fluctuations were similar in time course to those observed for LH but were of lesser magnitude (fig. 1). This daily rhythm of FSH was still present 3 to 4 days after ovariectomy (fig. 2), but the serum FSH concentrations in ovx females were significantly higher than the respective levels found in sham-ovx animals at 10.00 h ($P < .01$) and 15.00 h ($P < .005$).

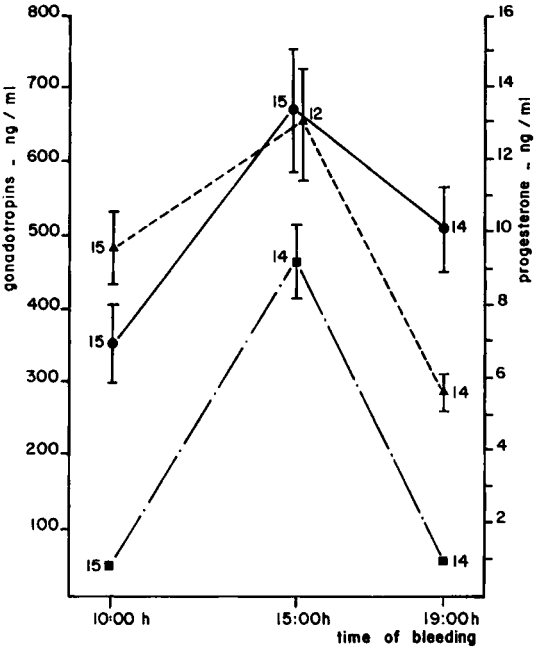

FIG. 1. — *Serum LH, FSH, and progesterone in « short-day » acyclic hamsters*

Values are expressed as means ± SEM. Since serum LH was undetectable in most of the samples collected at 10.00 h and 19.00 h, no SEM could be calculated for these points. The assay was capable of detecting 46 ng/ml serum. The number adjacent to each mean is the N for that group.

● progesterone
▲ FSH
■ LH

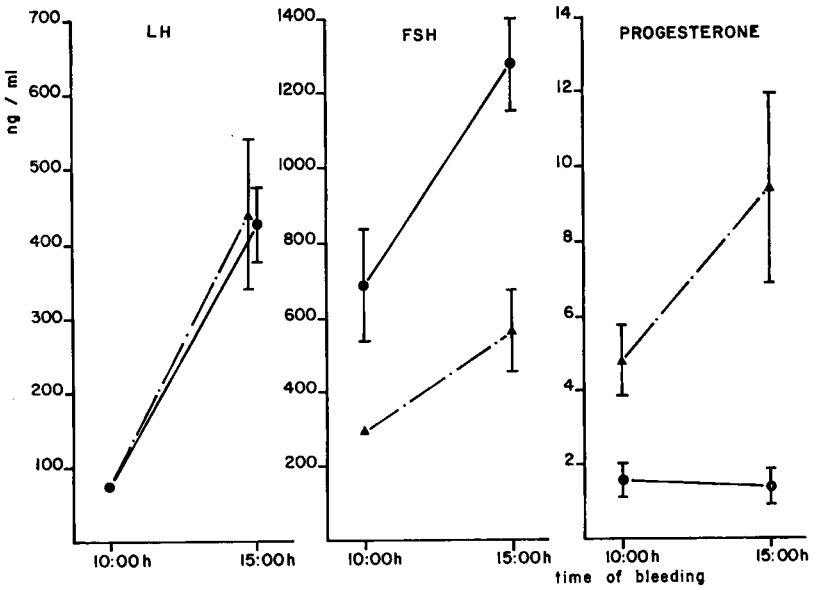

FIG. 2. — *The effect of ovariectomy in « short-day » acyclic hamsters upon the diurnal rhythm of gonadotropins and progesterone*

Values are expressed as means ± SEM. Serum LH was undetectable in most of the samples obtained at 10.00 h and FSH was undetectable in most of the samples collected from intact females at 10.00 h.

▲ sham-ovx
● ovx

Progesterone : Serum progesterone values were found to differ throughout the day ($P < .005$). Progesterone levels rose between 10.00 h and 15.00 h ($P < .005$) and then decreased between 15.00 h and 19.00 h ($P < .10$) (fig. 1). Ovariectomy resulted in lower progesterone levels at both 10.00 h ($P < .025$) and 15.00 h ($P < .01$) when compared to sham-ovx controls (fig. 2), and no afternoon rise was present in the ovx females. In each of the 6 sham-ovx hamsters progesterone levels increased between 10.00 h and 15.00 h ($P = .032$).

Effects of melatonin on cyclicity

Exogenous melatonin at dose levels of 25 μg daily and 2.5 μg daily inhibited cyclicity when administered at 15.00 h but not when given at 08.00 h (fig. 3). Injections of the oil vehicle or of 0.25 μg melatonin did not interrupt vaginal cyclicity.

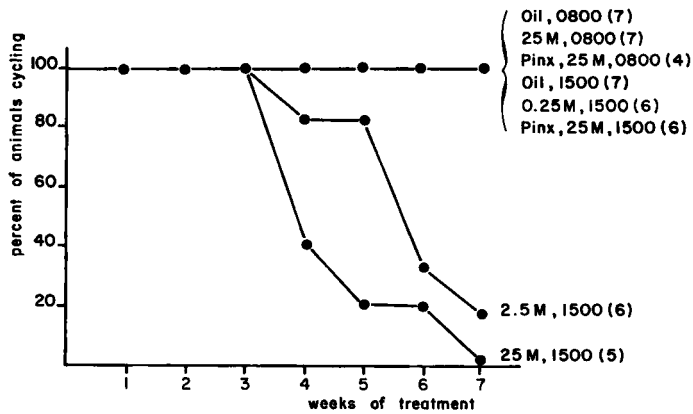

FIG. 3. — *Inhibition of estrous cyclicity by exogenous melatonin*

Female hamsters injected daily with 0.1 ml oil or 25 μg melatonin (25 M) at 08.00 h or with oil or 0.25 μg melatonin at 15.00 h all continued to show regular 4 day cycles throughout the 7 week treatment period. Females receiving 2.5 μg melatonin at 15.00 or 25 μg melatonin at 15.00 h became acyclic after 4-7 weeks with one exception. Pinealectomized (pinx) animals continued to cycle when given 25 μg melatonin daily at 08.00 h or 15.00 h. Numbers in parentheses indicate the N for each group.

Also, pinealectomized females continued to cycle after daily treatment with 25 μg melatonin given at either 08.00 h or 15.00 h. After 3 weeks of injections all the females were still cycling. Intact animals receiving the higher doses of melatonin at 15.00 h became acyclic only after 4-7 weeks of treatment. Similarly, male hamsters showed testicular regression when given 25 μg melatonin daily at 15.00 h (table 1). Histological examination of these testes revealed the absence of spermatozoa (Brown, unpublished data). Two of 5 males receiving 2.5 μg melatonin daily at 15.00 hhad regressed testes, but regression failed to occur in any of the animals given 0.25 μg melatonin. Oil injected controls and males given 25 μg melatonin at 08.00 h had large testes (with the exception of one male which had partially regressed testes following treatment with 25 μg melatonin at 08.00 h). Body weights were significantly increased in males receiving 2.5 or 25 μg melatonin daily at 15.00 h. In a

second study melatonin (25 μg/day) injections administered at 15.00 h induced testicular regression while injections given at 09.00 h had no effect on testicular weight (table 2).

TABLE 1

Testicular regression following single daily injections of melatonin in hamsters maintained on long days

(14 hours illumination daily) (1)

Injection regime	N	Body weight (g)	Testicular weight (mg)
Oil, 08.00 h	7	128.1 ± 2.0 (2)	3,556.2 ± 95.4
25 μg melatonin, 08.00 h	7	137.9 ± 2.9	3,294.3 ± 375.3
Oil, 15.00 h	6	134.6 ± 4.9	3,691.7 ± 94.7
25 μg melatonin, 15.00 h	7	159.1 ± 7.3*	628.1 ± 174.5**
2.5 μg melatonin, 15.00 h	5	157.6 ± 8.5*	2,561.4 ± 588.9
0.25 μg melatonin, 15.00 h	6	132.0 ± 7.7	3,313.1 ± 156.7

(1) Lights on 05.00 h - 19.00 h.
(2) Mean ± SEM.
* Significantly different from oil at 08.00 h and from oil at 15.00 h ($p < .05$).
** Significantly different from oil at 08.00 h and from oil at 15.00 h ($p < .001$).

TABLE 2

Testicular regression following daily injections of melatonin in hamsters maintained on long days

(13 hours illumination daily) (1)

Injection regime		N	Body weight (gms)	Testes weight (mg)
09.00 h	15.00 h			
oil	oil	7	126 ± 2 (2)	3,238 ± 141 (2)
25 μg melatonin	oil	6	138 ± 7	3,229 ± 85
oil	25 μg melatonin	6	139 ± 7	1,617 ± 287*

(1) Lights were on at 05.00 - 18.00 h daily.
(2) Mean ± SEM.
* Significantly different from both of the other groups ($p < .001$).

DISCUSSION

Daily rhythms in hormone levels have been reported in animals in various reproductive states. LAWTON and SMITH (1970) found a daily surge of LH in the afternoon in long term ovariectomized rats and a similar surge has been reported in

the ovariectomized hamster (GOLDMAN *et al.*, 1971). Other investigators have reported a daily surge of LH following estrogen treatment in the ovariectomized hamster (NORMAN and SPIES, 1974) and rat (LEGAN *et al.*, 1975). In the present study a state of acyclicity accompanied by a diurnal rhythm of gonadotropin and progesterone levels in the serum was found in female hamsters maintained on short days.

In the acyclic hamsters the pattern of low titers of LH and FSH early in the light period and elevated titers prior to the end of the light period was similar to the findings of SEEGAL and GOLDMAN (1975). In the present study ovariectomized females bled 3-4 days after surgery showed no change in the daily level or pattern of LH secretion when compared to their sham-ovx controls. In contrast, SEEGAL and GOLDMAN found that 3 ½ weeks after ovariectomy LH levels were more elevated during the afternoon although the diurnal pattern of secretion was similar to intact controls. Apparently, the latency for the elevation of the afternoon LH peak is more than 3-4 days. In both studies FSH levels were elevated at both bleeding times following ovariectomy. In contrast to the relative lack of effect of ovariectomy on serum LH concentrations in short-photoperiod hamsters, females maintained on long days show elevated LH levels within 3 ½ hours after ovariectomy (GOLDMAN *et al.*, 1971), and serum levels of both LH and FSH are greatly elevated 3 1/2 weeks after ovariectomy in both the morning and the afternoon (SEEGAL and GOLDMAN, 1975).

In addition to a daily surge of LH and FSH in short-day hamsters an elevation in the serum progesterone concentration was found to occur during the afternoon. The source of this progesterone appears likely to be ovarian interstitial tissue ; exposure to a short photoperiod induces a proliferation of the interstitial tissue in the hamster ovaries (REITER, 1968), and ovariectomy reduces the serum levels of progesterone and eliminates the afternoon surge in this hormone (fig. 2). BLAHA and LEAVITT (1970) found the enzyme Δ^5-3β-hydroxysteroid dehydrogenase, which is necessary for progesterone synthesis, in hamster ovarian interstitial tissue.

Although it is not known what factor(s) stimulates progesterone synthesis and release in the acyclic hamster, it seems reasonable to postulate that LH is involved. GREENWALD (1974) recently demonstrated that in cyclic hamsters LH stimulated both the synthesis and release of ovarian progesterone ; FSH was found to stimulate an increase in synthesis, but not release, of progesterone. In addition to the gonadotropins, it would be of interest to measure serum prolactin in the acyclic hamster since prolactin appears to stimulate synthesis and release of progesterone from the corpora lutea in the pseudopregnant rat (SMITH *et al.*, 1975). In the acyclic hamster it is likely, therefore, that LH or some combination of LH, FSH, and perhaps prolactin stimulates the production and release of ovarian progesterone.

REITER (1968) has reported atrophic uteri in light-deprived hamsters, suggestive of low levels of circulating estrogen. This finding, when combined with earlier observations of « intermediate » serum FSH concentrations and daily LH surges in acyclic females (SEEGAL and GOLDMAN, 1975), might be interpreted as evidence for decreased ovarian responsiveness to gonadotropins following exposure to short photoperiods. This possibility needs to be carefully reexamined. Nevertheless, even if a reduction in ovarian response to gonadotropins were apparent following light-deprivation, it would seem most likely that the ovarian changes might be secondary to the altered pattern of gonadotropin secretion.

While the findings of other workers suggest that a pineal hormone is involved in the phenomena described in this report, the identification of such a hormone and its mode of action remain to be discovered. Melatonin has been suggested as a likely candidate for the pineal hormone (WURTMAN *et al.*, 1968). However, REITER *et al.* (1974) has recently reported that implants of melatonin in beeswax *prevent* the testicular regression which normally ensues in the male hamster after prolonged exposure to a short photoperiod. Our observation of strongly rhythmic secretion of LH independent of the ovaries in the acyclic hamster raised a possibility which, perhaps, has not received adequate attention. Since the pineal itself is a highly rhythmic gland (KLEIN, 1974), it seems plausible that the effect of the pineal in mediating photic-induced gonadal regression may be related to a shift in phase of some pineal rhythm rather than to a simple increase in the secretion of an anti-gonadal hormone. For example, an interaction between a rhythmically secreted pineal hormone and a brain region with an independent rhythm might be responsible for seasonal cycles. Precedent for such a two-system interaction comes from the work of Meier and his colleagues (MEIER and MCGREGOR III, 1972). These investigators have described interactions between diurnal rhythms in adrenal corticosterone and prolactin, respectively, in controlling seasonal cycles in reproductive state and migratory behavior in sparrows.

The present observation that estrous cyclicity and testicular function was inhibited by melatonin administered at 15.00 h but unaffected by melatonin given at 08.00 h or 09.00 h lends support to the hypothesis that pineal diurnal rhythmicity may be a key factor in regulating seasonal reproductive rhythms in the hamster. However, the finding that exogenous melatonin was not effective in pinealectomized females suggests that this compound may act within the pineal rather than acting as a hormone.

Vasotocin has been found in pineal extracts from cows (CHEESMAN and FARRIS, 1970), and melatonin stimulated the release of vasotocin into the cerebrospinal fluid of the cat (PAVEL, 1973). Vasotocin has been reported to possess anti-gonadal properties in mice (PAVEL and PETRESEU, 1966 ; VAUGHAN *et al.*, 1974). In addition, preliminary data from our laboratory suggest that vasotocin (0.83-2.5 μg administered subcutaneously) is capable of inhibiting LH secretion in the male hamster (GOLDMAN, unpublished data). Thus, it may be that melatonin acts to stimulate (or inhibit) the release of vasotocin or some other pineal compound and that this may be the means by which melatonin exerts its « anti-gonadal » effects.

It is not clear whether the increase in body weight following afternoon injections of melatonin in male hamsters (table 1) was a primary effect of the indole or whether the alteration of body weight was secondary to the suppression of testicular function.

In summary, these results indicate that further work should concentrate on (1) determining the identity of the pineal hormone which has « anti-gonadal » properties, (2) studying the pattern of secretion of this hormone under various photoperiodic regimens and (3) determining the mechanisms of action of the pineal hormone.

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RÉSUMÉ

EFFETS DE LA PHOTOPÉRIODE ET DE LA MÉLATONINE SUR LA REPRODUCTION DU HAMSTER SYRIEN

Le Hamster est un animal à ovulation spontanée qui, dans la nature, présente une saison sexuelle.

La durée du jour (photopériode) apparaît être un facteur important dans la régulation de la saison sexuelle. Après 6-8 semaines d'exposition à des « jours courts » (photopériode 10 h) les femelles deviennent acycliques. Elles présentent cependant un rythme circadien de sécrétion de LH très marqué avec des décharges de LH dans l'après-midi. Les concentrations sériques de FSH et de progestérone montrent la même évolution journalière que la LH, mais moins importante. La castration diminue le taux de progestérone sérique et fait disparaître l'élévation de progestérone observée l'après-midi, mais le rythme circadien de sécrétion de LH et FSH n'est pas modifié.

Des injections journalières de mélatonine, sécrétée par l'épiphyse, inhibent les cycles d'ovulation chez les femelles maintenues en « jours longs » (photopériode 14 h) quand on l'administre 4 heures avant l'extinction de la lumière (15 h). Si on administre la mélatonine à 8 h ou si les femelles sont épiphysectomisées, on n'observe plus d'inhibition de l'ovulation.

On observe également des effets inhibiteurs de la mélatonine sur la reproduction du mâle qui dépendent du moment de la journée auquel on l'administre.

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