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Pineal Melatonin Content throughout the Hamster Estrous Cycle (40649)[1]

M. D. ROLLAG, H. J. CHEN, B. N. FERGUSON, AND R. J. REITER

The University of Texas Health Science Center at San Antonio, Department of Anatomy, 7703 Floyd Curl Drive, San Antonio, Texas 78284

The reproductive system of adult Syrian hamsters is extremely sensitive to photoperiodic effects (1). Upon exposure to short photoperiods female hamsters experience a dramatic decrease in uterine weight (2) and cease to exhibit vaginal changes associated with estrous cyclicity (3). Twenty to twenty-five weeks after initiation of short photoperiodic conditions gonadal function returns, even if there has been no change in photoperiodic cues (2). At this time hamsters are refractory toward short photoperiods and remain refractory until an intervening long photoperiodic exposure of at least 10 weeks has occurred (4, 5). The response of hamsters to short photoperiods is dependent upon an intact pineal gland (2). If the pineal gland is removed prior to exposure of hamsters to short photoperiods the gonads do not regress, whereas, if gonadal regression has already occurred, pinealectomy results in a rapid return in reproductive function. Daily injections of melatonin, a putative pineal hormone, can induce gonadal regression in pinealectomized hamsters (6, 7). Consequently, melatonin is considered by many investigators to be the pineal factor which mediates photoperiodic effects upon the reproductive system of this species. Recently, it has been reported that melatonin injections induce vaginal acyclicity in hamsters when administered on the evening of proestrus of each cycle for five cycles but not when administered on the evening of diestrus (8). Since data obtained from rats indicate that less melatonin is found in urine collected on Day 1 (proestrus) of the estrous cycle (9), it could be hypothesized that in long photoperiodic conditions the hamster pineal gland is inhibited by the preovulatory surge in estrogen secretion and that in short photoperiodic conditions the pineal gland becomes insensitive to estrogen feedback, an escape which results in gonadal regression. To test the validity of this hypothesis we have quantified pineal melatonin content throughout the estrous cycle of female hamsters maintained in a 14-hr-light:10-hr-dark photoperiod. If the hypothesis is correct, we would expect to observe a decrease in pineal melatonin content on the day preceding ovulation when estrogen concentrations are at a maximum.

Materials and methods. Adult female hamsters, 100–120 g, obtained from Lakeview Hamster Colony (Newfield, N.J.) were housed in a room with overhead fluorescent lighting (Sylvania Super Saver 35, F40W/RS/SS). Lights were turned on automatically at 0600 hr and off at 2000 hr (14-hr-light:10-hr-dark). Food and water were supplied *ad libitum.* Progression through 4-day estrous cycles was monitored for each hamster for a period of 1 month (10). At the end of this interval, 32 groups of seven to eight hamsters each were decapitated sequentially at 1000, 1400, 1800, 2200, 2400, 0200, 0400, and 0600 hr for 4 consecutive days. Hamsters were chosen so that representatives from each day of the estrous cycle were present at each sacrifice time. Those decapitations occurring at night were performed with the aid of a dim red light (25-W tungsten bulb behind a No. 1A Safelight filter, Kodak). Following decapitation, pineal glands were collected and placed into individual 1-ml aliquots of chilled 0.1 *M* sodium phosphate buffer, pH 7.0, containing 0.9% sodium chloride. The pineal glands were sonicated and stored as homogenates at −20°. Three weeks later, melatonin concentrations were quantified by radioimmunoassay of duplicate 200-μl aliquots from each 1-ml homogenate (11). Pineal melatonin concentrations were compared by analysis of variance.

Results. The quantities of melatonin in female hamster pineal glands throughout the

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estrous cycle are presented in Fig. 1. Values represent the mean ± SEM for eight hamsters during times of darkness and for seven hamsters during times of light. In the four radioimmunoassays utilized to obtain these values, control samples estimated to contain 171, 334, and 1006 pg/ml had respective intraassay coefficients of variation averaging 12.7, 6.8, and 9.6%. The respective interassay coefficients of variation were 14.9, 9.3, and 12.3%. The mean quantity of immunoreactivity in a 1 ng/ml solution of melatonin in assay buffer ranged between 951 and 1142 pg/ml. The least detectable concentration of melatonin ranged between 3 and 34 pg/ml.

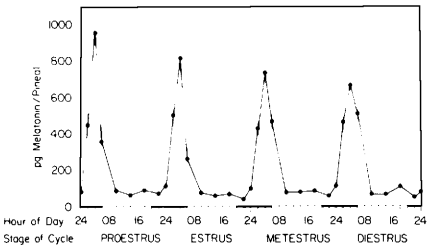

FIG. 1. Pineal melatonin content throughout the hamster estrous cycle. Values represent the mean ± SEM for eight hamsters during times of darkness and for seven hamsters during times of light. The solid bars on the time axis represent darkness in successive 14-hr-light:10-hr-dark photoperiods.

On each day of the estrous cycle a distinct rhythm in pineal melatonin content was observed. Mean melatonin concentrations ranged between 37 and 112 pg/gland throughout the day and during the first 4 hr of darkness. The highest mean melatonin concentrations, which ranged between 669 and 954 pg/gland, occurred at 0400 hr, 2 hr prior to the onset of light. Comparison of melatonin concentrations in pineal glands obtained at 0600 hr, just prior to light on, with those in pineal glands obtained at 0400 hr yielded a significant difference ($P < 0.001$). No significant difference could be found between values obtained at a particular time of day as a function of day of the estrous cycle. In particular, the F value obtained from analysis of samples collected at 0400 hr was 1.09 ($P > 0.2$).

Discussion. The results presented in this communication indicate that pineal melatonin production in hamsters is not inhibited by estrogen feedback in long photoperiodic conditions. Consequently, darkness-induced gonadal regression in hamsters cannot be explained by hypothesizing that short photoperiodic conditions result in an escape of the pineal gland from estrogen inhibition of melatonin synthesis. The data do not exclude hypotheses which depend upon 4-day cyclic changes in pineal melatonin production in hamsters maintained in short photoperiods. The lack of a difference in pineal melatonin production on different days of the hamster estrous cycle is similar to the results reported for sheep (12), but opposite to those reported for rats (9, 13) and humans (14). The reason for this difference is unknown. It is possible that pineal melatonin content, as reported here, does not accurately reflect secretion. This possibility seems unlikely since no data are available which suggest that melatonin is either stored or degraded within the pineal gland. Until such storage or degradation is demonstrated it can be assumed that all melatonin produced within the pineal gland is secreted and that pineal melatonin content accurately reflects secretory rates. This assumption is supported by data obtained from rats showing that pineal and serum melatonin concentrations have parallel peaks and troughs (15), and that photo-induced reduction in pineal melatonin content is closely paralleled by reduction in serum melatonin concentrations (16).

The circadian rhythm in pineal melatonin content of female hamsters reported in this study is of similar magnitude and duration as that reported for male hamsters maintained in the same photoperiodic conditions (17) and for female hamsters maintained in an 8-hr-light:16-hr-dark photoperiod (unpublished observations). The decrease in pineal melatonin content prior to the dark-to-light transition was found in male hamsters (17) and has been reported for rats (15). This decrease in pineal melatonin concentrations prior to exposure to light supports the contention that melatonin production is a consequence of an endogenous circadian rhythm.

Summary. A circadian rhythm in pineal melatonin content persisted throughout the estrous cycle of female hamsters maintained in a 14-hr-light:10-hr-dark photoperiod. Peak melatonin concentrations occurred 8 hr after the onset of darkness and were of similar

duration and magnitude on each day of the reproductive cycle. These results do not support the hypothesis that pineal melatonin production in hamsters is inhibited by estrogen feedback.

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