PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 169, 416–419 (1982)

Variation in Pineal Melatonin Content during the Estrous Cycle of the Rat (41368)

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Abstract. Pineal levels of melatonin exhibit a circadian rhythm in the rat. To determine if this rhythm varies during the estrous cycle, adult rats were sacrificed every 2 hr from 2000 hr (prior to lights off) until 0800 hr (2 hr after lights on) throughout each day of the 4-day estrous cycle. Pineal glands were assayed for melatonin by radioimmunoassay. A significant circadian rhythm in the pineal content of melatonin was evident each day of the cycle with peak levels exhibited during the dark phase. There were significant differences in titers of melatonin present at 0200, 0400, and 0600 hr between various stages of the cycle. In general, highest nighttime levels occurred during the evenings of metestrus and diestrus, with levels at a minimum on the evening of estrus. These data indicate that the circadian pineal melatonin rhythm is a function of estrous cycle stage in the rat, and, thus, important differences exist in the pineal biosynthetic dynamics of this species relative to those of seasonal breeders such as the hamster and sheep. Additionally, the results suggest that hydroxyindole-*O*-methyltransferase, and not serotonin *N*-acetyltransferase, is the regulatory enzyme responsible for these estrous stage differences in pineal melatonin content.

The pineal gland is widely accepted as a regulator of mammalian reproduction due to its ability to influence gonadal size and function, estrous cyclicity, and pregnancy, as well as other sexual parameters (1). Since the pineal gland functions as an integral component of the neuroendocrine–reproductive axis, it is reasonable to assume that it, in turn, might be regulated by feedback controls determined by the prevailing reproductive status of the animal. Indeed, pineal function and, in particular, indole metabolism, have been shown to be modified by castration and the administration of gonadotrophins and steroids associated with reproduction (2–4). Another approach to this question has been to examine pineal activity as a function of the estrous cycle, consisting of naturally occurring alterations in female reproductive status. Pineal activity is frequently evaluated in terms of the level of the hormone melatonin (*N*-acetyl-5-methoxytryptamine).

Quay (5), spectrophotofluorometrically measuring rat pineal melatonin levels, reported that early evening values tended to vary with the day of the cycle, although these changes did not prove significant. Radioimmunoassayable melatonin in rat urine (6) and the serum of normal adult women (7) has also been shown to be a function of the female reproductive cycle. However, day and nighttime levels of melatonin were not a function of reproductive state in the serum of ewes (8) or pineals of hamsters (9). Similarly, morning levels of blood melatonin did not vary during the menstrual cycle of the monkey (10).

A possible estrous cycle variation in pineal melatonin synthesis has also been analyzed in terms of the activities of the enzymes serotonin *N*-acetyltransferase (NAT) and hydroxyindole-*O*-methyltransferase (HIOMT), responsible for the conversion of serotonin to melatonin. Although HIOMT activity in the rat pineal gland has been reported to depend upon estrous cycle state (11–13), NAT activity is apparently not altered by this factor (14, 15).

To date, the circadian rhythm of pineal melatonin has not been well characterized as a function of estrous cycle stage in the rat. Such previous studies have been inconclusive since only a single time was examined (5), levels were determined in nighttime urine (6), or enzyme activities were measured rather than directly monitoring melatonin levels. A variation in the pineal

0037-9727/82/030416-04$01.00/0

melatonin rhythm (i) would suggest a possible susceptibility to feedback from the reproductive axis, (ii) might indicate possible neural or hormonal factors responsible for alterations in pineal activity, and (iii) would establish a potentially important difference between pineal function in the rat and hamster, two laboratory species frequently utilized in pineal research.

Materials and Methods. Two hundred and seventy-two young adult Sprague–Dawley-derived female rats (200–225 g) were obtained from Simonsen Laboratories, Inc. (Gilroy, Calif.) and placed in a 14L:10D lighting cycle with lights on at 0600 hr. Food and water were supplied *ad libitum*. Vaginal smears were taken daily to monitor estrous cycles. Three weeks after arrival, rats exhibiting at least three successive 4-day cycles were sacrificed during each stage of the cycle at 2000, 2200, 2400, 0200, 0400, 0600, or 0800 hr. All animals were sacrificed over a 2-day period. Rats were chosen such that each group (representing a particular cycle stage and time) consisted of 8 to 12 rats. A dim red light (25-W tungsten bulb behind a No. 1A Safelight filter, Kodak) was used to aid decapitation during the scotophase. Pineal glands were obtained at autopsy and placed in 1 ml of a chilled 0.1 *M* sodium phosphate buffer containing 0.9% sodium chloride. The glands were disrupted by sonication and the resulting solutions frozen at −20° until the time of assay. Melatonin was determined by radioimmunoassay (16) of 200-μl duplicate aliquots of each pineal sample and were compared by an analysis of variance followed by a Student/Newman–Keuls test for differences among multiple means. Data are expressed in the present report as melatonin content (pg melatonin/pineal).

Results. For convenience of discussion each "day" of the estrous cycle is defined to include a single light and dark period, extending from 0600 hr of one day to 0600 hr of the next day (Fig. 1). By this scheme, ovulation would occur near the middle of the dark period of the day designated "proestrus." On each day of the estrous cycle, a pronounced circadian rhythm in pineal melatonin content was evident (Fig. 1). On any particular day, peak values during the dark were significantly greater than values at 2000 hr (immediately prior to lights off) or 0600 hr (immediately prior to lights on) ($P < 0.001$). In addition, a 4-day rhythm in nocturnal melatonin content was evident. In general, the melatonin content of pineals obtained during darkness significantly decreased between the days of diestrus and estrus, and was again elevated at the next metestrus. At 0200 hr, the diestrous melatonin content was significantly greater than that of estrus ($P < 0.025$). Two hours later, at 0400 hr, the metestrous melatonin content was significantly greater than that of estrus, diestrus, and proestrus ($P < 0.005$, $P < 0.025$, and $P < 0.025$, respectively). At 0600 hr, the metestrous melatonin content significantly exceeded the levels during proestrus and estrus ($P < 0.05$ and $P < 0.01$, respectively) and the diestrous level was greater than that of estrus ($P < 0.05$). All of these differences were observed at 0200 hr or later; prior to this time, values did not vary with cycle stage.

Discussion. The rat pineal gland exhibited a circadian rhythm in melatonin content which varied with the stages of the es-

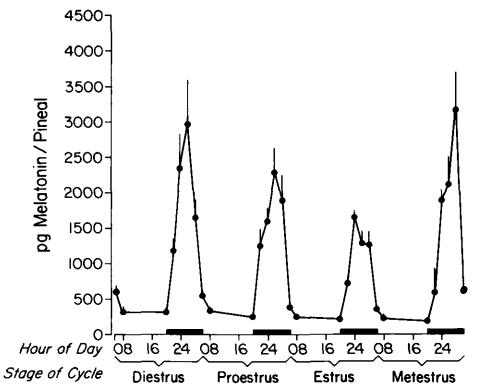

FIG. 1. Melatonin content in pineals of adult female rats sacrificed every 2 hr from 2000 hr of one day to 0800 hr of the next, as a function of estrous cycle stage. Each point represents mean of values from 8 to 12 animals and lines represent standard errors. Dark bars indicate periods during which lights were off. Note that each estrous cycle stage includes one light and dark period and is 24 hr in length.

trous cycle (Fig. 1). In general, nighttime values were highest on metestrus and diestrus and decreased over proestrus and estrus. Since values did not vary with the day of the cycle until 0200 hr and later, it appears that the initial rise in pineal melatonin is relatively independent of cycle stage. This finding is consistent with data showing no significant difference in melatonin levels 3 hr into the dark phase on the various days of the cycle (5); in this earlier study, however, a "trend" for melatonin content to be reduced during diestrus was noted, contrary to the present findings.

Perhaps most enlightening is analysis of the present data in light of previously published data involving measurement of pineal enzyme activities. Pineal melatonin dynamics in relation to estrous cycle stage have often been investigated in terms of the enzymes NAT, responsible for the conversion of serotonin to *N*-acetylserotonin and HIOMT, which catalyzes the final step in melatonin synthesis. Both of these enzymes exhibit a circadian rhythm in pineal tissue similar to that of melatonin (17, 18). Attempts to correlate rat NAT activity with estrous cycle stage have been unsuccessful to date (14, 15); it appears that this rhythm is independent of the naturally occurring alterations in reproductive state during the female cycle. However, HIOMT activity has been frequently reported to depend upon estrous cycle state (11–13).

Wurtman *et al.* (11) described morning levels of HIOMT to be lower on proestrus and estrus than on metestrus and diestrus. In more detailed studies where HIOMT levels were measured at several times throughout a 24-hr period, peak nighttime values were decreased during proestrus and at a minimum on estrus, relative to the other nights of the cycle (12, 13). (Note that for the sake of comparison, all data are discussed in terms of the estrous cycle days defined in the present study; each "day" of the cycle begins at the onset of light and includes one period of light and dark.) Wallen and Yochim (12) suggest that the complex waveform of HIOMT activity throughout the estrous cycle is best described as consisting of two component rhythms of different frequencies; when the component rhythms are out of phase (estrus) a "damped" oscillation occurs. In this analysis, the mean rate of enzyme activity throughout the cycle is constant while the amplitude of oscillation around this mean is altered during the estrous cycle. Correlation of these previous studies of pineal enzyme levels during the estrous cycle with the melatonin levels reported here suggests that (i) although NAT is often considered the key regulatory enzyme for melatonin biosynthesis (19), HIOMT is more likely the enzyme responsible for the pattern in melatonin content determined in the present study and, (ii) the nighttime activity of HIOMT (rather than the mean daily rate of enzyme activity) is precisely correlated with the nighttime melatonin content of the pineal during the estrous cycle. This first observation is in keeping with the hypothesis of a dual regulation of melatonin synthesis (20), predominantly neuronal control via NAT and hormonal control via HIOMT.

The mechanism by which melatonin content and, presumably, HIOMT activity is altered during the estrous cycle can only be speculated upon at this time. Both pineal HIOMT activity and melatonin synthesis are altered by treatment with exogenous steroids or castration (21); however, the basic estrous cycle pattern of rhythmicity does not appear to be dependent on the presence of the ovaries (3). Possible explanations for the correlation of pineal activity with the estrous cycle are (i) the reproductive system and pineal gland are independently under the control of the same internal neural oscillator, or (ii) a complicated and as yet undefined interplay of hormonal factors (possibly ovarian, pituitary, and/or hypothalamic) are responsible for the definitive form of the pineal melatonin rhythm. It is certain, however, that variations in pineal activity are not *responsible* for female estrous changes since pinealectomized animals still cycle regularly (1).

The data of many studies in which estrous or menstrual cycle dependence of the pineal melatonin rhythm has been investigated are inconclusive due to the selection of only a single daily time point (5, 7, 10).

Detailed determinations in the sheep (8), the hamster (9), and now the rat reveal a marked difference among species. The sheep and hamster, both seasonal breeders in their natural habitat, exhibit no estrous cycle variation in the circadian melatonin rhythm. One might hypothesize that in these former species, a delicate control system of checks and balances is operative and able to "damp out" daily fluctuations in pineal activity; the primary goal of pineal function in these animals would be long-term seasonal control of reproduction. By contrast, the inbred laboratory rat, known to be relatively insensitive to the effects of pineal hormones, may have additionally lost a "fine-tune" control of pineal activity. In a species no longer particularly sensitive to the actions of the pineal gland, the regulation of the gland would be of little physiological significance and might be lost.

To date, an estrous cycle dependence of the pineal melatonin rhythm appears to be clearly evident only in the rat. Knowledge of the mechanism by which this pattern is accomplished, the possible physiological implications of these alterations, and the possible presence of estrous cycle dependency in other species awaits further study.

The authors wish to express their gratitude to Bonnie N. Ferguson, Diep T. Dinh, and Joachim Huerter for their excellent technical assistance.

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Received September 9, 1981. P.S.E.B.M. 1982, Vol. 169.