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Effect of Thyroidectomy on Rhythmic Gonadotropin Release (39135)[1]

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The cyclic nature of adenohypophysial activity has become well established in recent years. Characteristically, the concentration of adenohypophysial hormones in the blood fluctuate in an episodic, or pulsatile, fashion (1, 2) as well as with a daily rhythm (3); pituitary content of hormone can show daily fluctuations also. These daily changes are generally synchronized with the light–dark cycle of the environmet. In the absence of the light–dark oscillations, or other synchronizing forces, the hormones maintain the rhythm but the frequency usually deviates somewhat from the 24 hr.

Although the significance of these rhythms is not understood well, evidence is accumulating that neuroendocrine rhythms are important for producing patterns in peripheral hormone levels necessary for evoking specific physiological events. Normal or abnormal functions could therefore be a result of synchronization or desynchronization of rhythms of interacting hormones. Subtle changes in the phase angle between these rhythms may produce marked changes in endocrine function (4, 5). Any agent that can alter these phase angles therefore gains importance in the same sense as does a permissive or augmenting agent. Increasing evidence implicates thyroxin (4) as a phasing agent for several rhythmic endocrine functions. In the present study, the effect of loss of thyroid hormone on rhythmic changes in serum gonadotropins in male rats was studied.

Materials and Methods. Adult Sprague–Dawley (Charles River, CD) rats were housed (two per cage) under conditions of controlled lighting (fluorescent illumination from 4 AM to 6 PM) and temperature (24 ± 2°) for 40 days prior to the study. Thyroidectomy (TE) was performed surgically at approximately 40 days of age. Following surgery, TE rats were housed indiscriminately with intact or TE animals. Three days prior to the study, rats were transferred to individual cages and handled daily in order to familiarize animals with entry to animal quarters, cage opening, and removal from the cage. To further standardize conditions, the animal quarters were locked and not entered during the 18 hr preceding the experiment. In addition, all treatment and collection procedures, assigned and carried out according to a randomized block design, were done outside the animal quarters. Purina laboratory chow and tap water were available to the animals at all times.

The methods used to assess nonstress serum levels of LH, FSH, prolactin, and corticosterone were similar to those described previously (6). Nonstress blood samples were obtained by removing rats individually from the animal quarters to an adjacent preparation room where they were rapidly decapitated (<20 sec following cage opening). Blood from the trunk was collected and centrifuged following clot formation. Serum was removed, frozen, and stored for subsequent analysis. Individual serum samples were assayed for LH, FSH, prolactin, and corticosterone concentrations. Immediately after decapitation, the ventral prostate and seminal vesicle glands were removed, cleaned, and weighed to the nearest 0.1 mg.

Radioimmunoassays. Serum samples were analyzed for LH, FSH, and prolactin using double antibody radioimmunoassays. The materials used were provided by the NIAMDD-Rat Pituitary Program. Anti-rabbit gamma globulin was prepared locally in sheep. Details of the procedure used have been published (7). Concentrations of go-

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nadotropins were expressed in ng/ml on the basis of the standards supplied (RP-1).

Corticosterone assay. Serum concentrations of corticosterone were determined with the fluorometric method of Guillemin *et al.* (8).

Statistical probabilities were derived from analysis of variance and Student's *t* test.

Results. LH. A rhythm in nonstress LH levels similar to that reported previously from this laboratory (6) was observed during the experimental period (F (7,50) = 2.94, $P < 0.01$). Circulating LH levels began to increase at 8 PM and peak levels occurred at 11 PM (Fig. 1). Comparison of the highest mean during the 24-hr period (37.8 ± 5.0 at 11 PM) with the lowest mean (23.7 ± 1.8 at 11 AM) showed a 60% increase in serum LH levels. Differences between the overall mean (27.5 ng/ml) and trough and peak values were also quite significant, P being < 0.01 for both comparisons.

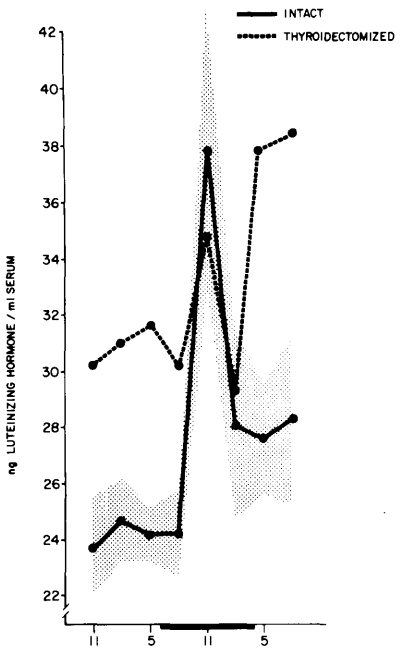

FIG. 1. Comparison of the circadian patterns of nonstress and stress levels of serum LH intact and thyroidectomized male rats. Here and in Fig. 2, the continuous and interrupted lines indicate the mean LH values. The stippled area indicates the standard error and the horizontal black bars indicate periods of darkness.

As illustrated in Fig. 1, TE markedly altered serum LH levels. The overall LH mean in TE rats (32.8 ng/ml) was higher than that of controls ($P < 0.01$) and significant periodicity in circulating LH levels appeared to be absent in the TE rats (F (7, 49) = 1.62, $P > 0.05$). Highest values occurred at 5 and 8 AM, 3 to 6 hr later than peak LH times in controls; however, LH values at 5 and 8 AM were not significantly higher than those at 11 PM, nor were they higher than several of the earlier time points.

Prolactin. As expected, intact male rats demonstrated circadian periodicity (F (7,52) = 4.45, $P < 0.01$) in serum prolactin levels (Fig. 2). Peak (23.9 ng/ml) and trough (8.4 ng/ml) levels occurred at 11 PM and 11 AM, respectively, and the latter were significantly different from the overall mean (13.2 ng/ml). Comparison of peak and trough levels indicated a 180% increase in serum prolactin levels.

Serum prolactin levels in TE rats are illustrated in Fig. 2. As indicated, both the level and the phase of the periodicity in circulating prolactin levels were altered 40 days after surgery. The overall mean of 9.3 ng/ml was less ($P < 0.01$) than that of intact ani-

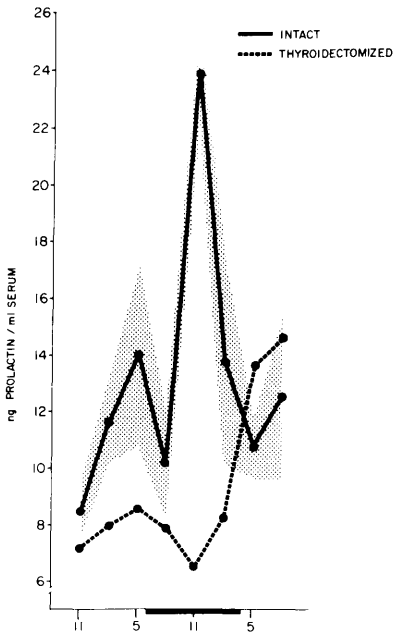

FIG. 2. Comparison of the circadian patterns of nonstress and stress levels of serum prolactin in intact and thyroidectomized male rats.

mals and peak prolactin values occurred during the early part of the light phase of the lighting schedule. Analysis of variance indicated the observed periodicity to be real (F (7,49) = 3.96, $P < 0.01$) and two-way analysis of variance showed a significant interaction between treatment and time (F (5,14) = $P < 0.01$).

FSH. Unlike LH and prolactin, serum FSH levels in control animals (Table I) did not fluctuate with a circadian periodicity (F (7,55) = 0.65, $P > 0.05$). The highest (192 ng/ml) mean value, which occurred at 8 PM, was not statistically different from the overall mean (183 ng/ml) nor was it different from the lowest mean (171 ng/ml) which occurred at 5 AM.

As in the case of intact animals, TE rats did not show a periodicity in serum FSH levels (F (7,46) = 1.93, $P > 0.05$). However, TE animals did show increased FSH levels (Table I). The 24-hr mean (202 ng/ml) was greater ($P < 0.01$) than that of the intact group. Additionally two-way analysis of variance showed significant interaction between treatment and time (F (7,101) = 2.36, $P < 0.01$).

Corticosterone. The results of measurement of corticosterone in these same serum samples are illustrated in Fig. 3. As expected, significant periodicity in nonstress serum corticosterone levels occurred during the 24-hr light–dark schedule (F (7,57) = 7.41, $P < 0.01$) and as in previous studies (6, 9), peak levels occurred at 5 PM just prior to the onset of the light-off phase. The overall mean was 11.5 μg/100 ml.

TABLE I. DAILY VARIATION IN SERUM FSH (NG/ML) LEVELS IN INTACT AND THYROIDECTOMIZED (TE) MALE RATS.

	Control	TE
11 AM	181 ± 6*	189 ± 10
2 PM	179 ± 5	208 ± 9
5 PM	190 ± 12	210 ± 14
8 PM	191 ± 8	169 ± 6
11 PM	180 ± 10	194 ± 13
2 AM	191 ± 10	192 ± 11
5 AM	171 ± 10	229 ± 29
8 AM	176 ± 11	223 ± 8
24 hr	188 ± 4.2	202 ± 5.4**

* Mean ± S.E.

** $P < 0.01$ compared to controls.

Serum corticosterone levels in TE rats showed a 24-hr rhythm (F (7,50) = 4.44, $P < 0.01$) similar to that of intact rats (Fig. 3). Neither the phase nor the level was different from that of control animals. Peak levels occurred at 5 PM ad the overall mean was 9.7 μg/100 ml. Two-way analysis of variance did not show a significant interaction between time and treatment (F (7,106) = 2.02, $P > 0.05$).

Seminal vesicle and ventral prostate. Although the wet weight of the ventral prostate of intact (F (7,56) = 0.46, $P > 0.05$) and TE (F (7,50) = 1.4, $P > 0.05$) rats did not vary significantly during the 24-hr experimental period, in both groups the highest weights were recorded during the late morning or early afternoon; the lowest weights occurred during the first part of the dark period.

Similar findings were observed for seminal vesicle weights in that both groups showed similar patterns in weight fluctuations. However, the weight fluctuations of the TE animals were significant (F (7,48) = 2.57, $P < 0.05$). The highest mean for intact animals (567 mg) occurred at 11 AM, the lowest (437 mg) at 11 PM, and the difference was significant at the 5% level. High (472 mg) and low (324 mg) values for TE rats occurred at 11 AM and 2 AM, respectively, and they were different ($P < 0.05$). In both cases the prostate and seminal vesicle weights of TE rats were considerably smaller ($P < 0.01$) than those of control animals.

Discussion. Allowing for subtle differences in environmental conditions, the temporal and quantitative aspects of the LH

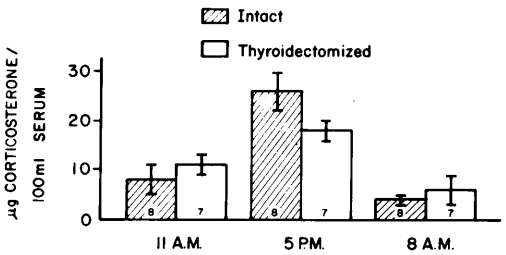

FIG. 3. Effect of thyroidectomy on nonstress serum corticosterone levels. In this illustration, number of animals is indicated at the base of the columns; vertical lines indicate standard error.

and prolactin rhythms in the present study are similar to those reported previously (6). Serum LH and prolactin levels began to increase at 8 PM and peak levels occurred midway into the dark period, i.e., 11 PM.

In contrast to earlier findings, significant periodicity was not observed in serum FSH levels. Johnson (10) reported 24-hr fluctuations in serum FSH levels and data collected in this laboratory (3) have suggested that circulating FSH varies with a daily periodicity. However, unlike LH and prolactin, the FSH rhythm has not been reproducible. Although the explanation for these observation differences are not known, the data indicate that FSH, for whatever reason, is not entrained to the light–dark schedule and/or does not fluctuate with a circadian periodicity. The latter observation is difficult to reconcile with the presence of a common LH–FSH releasing hormone since serum LH levels vary with a predictable circadian frequency.

While it is generally recognized that an interrelationship exists between the neuroendocrine mechanisms involved in maintenance of adrenal, gonadal, and thyroidal functions, specific information regarding the nature of such interactions remains limited. In the female rat, removal of the thyroid gland or blockade of thyroid hormone synthesis by feeding antithyroid drugs results in markedly reduced pituitary–ovarian function. Estrous cycles become irregular (11) and several indirect indices of ovarian function such as ovarian weight (11, 12, 13), number of follicles (14), number of corpora lutea (14) and number of eggs ovulated (15, 16) are decreased. Concomitant with decreased ovulation number was a decrease in blood and pituitary levels of LH, FSH, and growth hormone (16).

In the male rat, thyroidectomy appears to have little or no effect on testicular function. Hess (17) and, more recently, Vilchez-Martinez (18) showed thyroidectomized male rats to have normal gonadal and secondary sex organ weights along with normal testicular morphology (18). In the present study, prostate and seminal vesicle weights of thyroidectomized rats were less than those of intact controls. However, when the data were expressed as relative weights, the sex organ weights of thyroidectomized animals were not different from controls.

Our finding that the 24-hr mean of both LH and FSH in TE rats was increased was somewhat surprising since Vilchez-Martinez (18) recently reported that intact rats had a higher pituitary LH content than thyroidectomized animals. While it is possible that the elevated LH levels reflected a cross reaction of the LH-antibody with TSH, Monroe, Parlow, and Midgley (19), in the development of the assay system, reported that variations in the LH/TSH ratio over a range of 3400-fold failed to alter the indices of discrimination for the assay of LH. Thus, it would appear that TSH did not interfere with the RIA of LH but of course that was not actually verified in the present study. Since increased FSH levels cannot be similarly accounted for by hormonal cross reactivity within the radioimmunoassay system, the FSH data must be taken to indicate an overall increase in circulating FSH. Nevertheless, it should be noted that at any given time point the differences between control and thyroidectomized groups were relatively small and were not always significantly different.

Reports from several laboratories have indicated that circulating prolactin levels are significantly increased after thyroidectomy. Ieiri (20) reported that *in vitro* prolactin synthesis by rat anterior pituitaries was increased by prior thyroidectomy, and suppressed by T_3 treatment. Debeljuk *et al.* (21) found that treatment of sheep with triiodothyronine lowered resting prolactin levels and greatly inhibited the prolactin release normally observed with TRF administration. Clinical studies (22, 23) indicate that TRF markedly increases prolactin secretion in humans. Thus, the finding that thyroidectomized rats had decreased prolactin levels was not expected. However, consistent with our data, Chen and Meites (24) observed that chronic treatment of rats with T_4 resulted in increased pituitary prolactin content. Taken together, these observations suggest that considerable species variation exists in the ability of TRF to induce prolactin secretion. Whereas TRF may be extremely potent in stimulating the

secretion of prolactin in humans and sheep, its physiological role as a releasor of rat pituitary prolactin is questionable.

The temporal and quantitative aspects of the corticosterone data are similar to those reported previously (9). These data not only provide indirect evidence for the temporal aspects of ACTH release but also provide a well-established standard with which the rhythmic activity of other hormones can be compared.

Although the functional importance of these rhythms is not completely known, there exists evidence that neuroendocrine rhythms are involved in producing the peripheral patterns necessary for evoking specific physiological events. For example, in birds the time of greatest release of prolactin from the pituitary is important in regulating several seasonal physiological conditions (4). Depending upon its temporal relations with corticosterone, prolactin may induce fattening, loss of fat, cropsac proliferation, or a combination of all of the above. Similar findings have been reported for the rat (5). While prolactin augmented the steroidogenic action of LH on the testes of hypophysectomized male rats at whatever time it was given, maximal response was obtained when both hormones were injected 1 hr into the dark period. When prolactin and LH were given early in the light period, the testicular weight increase found with LH alone was inhibited. Thus, it appears that small changes in the phase angle between rhythms may produce marked changes in endocrine functions even though the 24-hr mean values are not altered. Whether the phase shift observed in prolactin and suggested for LH secretion resulted in altered neuroendocrine function is not known since neither gonadal function nor sex-related behavior patterns were evaluated. The data do, however, indicate the tenuousness of conclusions based on information obtained at a single time point. Differences between control and experimental groups using single time point sampling may reflect a phase shift in the secretory pattern and not an altered level of secretion. As indicated in Fig. 2, a single time point could be chosen to suggest that thyroidectomy results in increased, decreased, or no change in serum prolactin levels. Whether the periodicity observed in prolactin (and perhaps LH) levels in thyroidectomized animals represents a true phase shift or a free-running rhythm is not known. Of importance is the finding that the level of, and the phase angle between, these rhythms is markedly influenced by pituitary–thyroid function.

Summary. Nonstress blood samples were obtained from intact and thyroidectomized (TE) male rats at 3-hr intervals over a 24-hr period via rapid decapitation. The animals were thyroidectomized when 40 days old and used 6 weeks later. Intact animals showed periodicity in serum LH ($P < 0.01$) and prolactin ($P < 0.01$). Both gonadotropins began increasing after 8 PM and peak levels occurred at 11 PM. In contrast, 24-hr periodicity was not observed in serum FSH. Corticosterone levels in these same serum samples showed the expected circadian periodicity. After TE, the 24-hr pattern in all gonadotropins was altered significantly. Serum LH increased ($P < 0.01$) and circadian periodicity appeared to be absent. FSH and prolactin levels were increased and decreased, respectively ($P < 0.01$), with serum prolactin showing a 9-hr phase shift. Prolactin began increasing at 2 AM and reached a peak at 8 AM. Corticosterone in TE animals showed a 24-hr rhythm similar to that of intact rats. These findings confirm our previous observations that nonstress serum LH and prolactin levels fluctuate with a 24-hr periodicity and suggest that the level of, and the phase angle between, these rhythms is markedly influenced by pituitary–thyroid activity.

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