DIURNAL VARIATIONS
OF PLASMA AND PITUITARY THYROTROPHIN
IN ADULT MALE AND FEMALE RATS

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ABSTRACT

Male and female rats fed a low iodine diet for 20 days were used to study the diurnal variations in resting levels of plasma and pituitary TSH concentration using a highly sensitive radioimmunoassay. Sex differences in the fluctuations in plasma TSH levels and in amount of TSH in the pituitary gland were observed. The daily fluctuations of plasma TSH were characterized by two peaks that occurred in males at 6 a.m. and at 3 p.m., while in females the peaks were delayed until 9 a.m. and 7:30 p.m. Moreover, in the females the morning and the afternoon peaks were of the same intensity while in the males the afternoon peak that occurred just before the onset of darkness was much greater than the morning peak. There was a fall in TSH content of the pituitary in the male rats at 6 a.m. and also in the afternoon just before the onset of darkness. Thus, the diurnal variations in the plasma and pituitary TSH levels were related in male rats. In the females, however, the pituitary TSH concentration did not reflect the changes observed in the plasma TSH levels. The level of plasma PBI did not appear to be responsible for the fluctuations in plasma TSH concentration. It is suggested that the main mechanism for the control of the circadian rhythm of TSH might be related to a high activity at night.

Recent studies (Jolín & Tarín 1974; Pallardo et al. 1976) on thyroid function in male rats have shown that the rate of uptake of $^{131}$I and absolute iodine, the formation of labelled thyroxine and triiodothyronine, and the release of
and \(^{127}\text{I}\) is augmented in the thyroid gland in the afternoon and evening as compared to other times of the day. Thus the daily fluctuations in the activity of the thyroid gland might be related to variations in circulating TSH levels during the day; there is a rise in plasma TSH concentration at 3 p.m. compared to the levels observed at 9 a.m. and 1 p.m. (Pallardo et al. 1976). In order to investigate further the relationship between thyroid gland function and TSH levels, we determined the plasma and pituitary concentration of TSH, plasma PBI and thyroid iodine content in male and female rats at more frequent intervals than those used in previous studies.

**MATERIALS AND METHODS**

Adult male and female Wistar rats were used. They were housed 10 or 20 rats per cage in a temperature controlled room (22\(^\circ\)C) with a 12 h light-dark cycle (lights on 7 a.m. to 7 p.m.) for 20 days before experiments were begun. They were fed a low iodine diet (LID) (John et al. 1968) and drank distilled water *ad libitum*. The initial body weight of the animals was approximately 80–110 g, and it ranged between 180–245 g in males and between 110–180 g in females at the time of the experiments.

The rats were handled daily, beginning 10 days before experiments in order to minimize stress as far as possible on the test day. On the test day, 9 or 10 unanaesthetized male and female rats were decapitated with a guillotine at 9 a.m., 12 noon, 1:30 p.m., 4:30 p.m., 6 p.m., 7:30 p.m., 9 p.m., midnight, 5 a.m. and 6 a.m. the following morning. The rats were killed in this way to avoid stress which occurs with most blood withdrawal methods (Kokka et al. 1972). The trunk blood was collected into heparinized beakers, transferred to tubes and centrifuged immediately. Samples of individual plasma were frozen at -20\(^\circ\)C for analysis of PBI and TSH.

![Fig. 1](https://Example.com/figure1.jpg)

**Fig. 1.**

Diurnal variations in plasma TSH of male and female rats fed a low iodine diet (LID) for 20 days. Lights on from 7 a.m. to 7 p.m. Open circles = experiment A. Filled circles = experiment B.
Fig. 2.

Diurnal variations of pituitary TSH content of male and female rats. Experimental conditions and symbols as described in the legend to Fig. 1.

The thyroid gland and the pituitary gland were removed, dissected and weighed. Each pituitary gland was homogenized in 1 ml physiological saline and stored at −20°C until TSH radioimmunoassay was carried out.

Plasma PBI and thyroid iodine content were determined by a Zak procedure as modified by Benotti & Benotti (1963). The plasma and pituitary TSH concentration were measured by radioimmunoassay, using the double-antibody technique. The NIAMD-kit supplied by the National Institute of Arthritis and Metabolic Disease, Rat Pituitary Program, NIH was used for this purpose. Two dilutions of at least duplicate samples of each plasma or pituitary sample were used. The results were expressed in terms of the NIAMD-kit TSH-I-1 standard. The index of precision (λ) of the assays used for the data of TSH were 0.089 (Fig. 1, experiment A), 0.062 (Fig. 1, experiment B), 0.074 (Fig. 2, experiment A) and 0.071 (Fig. 2, experiment B).

All results are given as mean values ± sn. Statistical comparison between two groups or several groups was made by Student’s t-test or Turkey’s multiple comparison test as modified by Snedecor (1956), respectively. The entire experiment was carried out twice, once in April, 1974 (Experiment A) and again in July, 1975 (Experiment B).

RESULTS

Fig. 1 shows the variations in plasma TSH concentration observed during the day and night in two groups of male and female rats. The daily pattern of plasma TSH in each sex was characterized by two peaks. Some differences,
however, were found in the peak times and peak levels between the sexes. In males, the peak level occurred at 3 p.m. when it was 2–3.5 times greater than the mean of all other values for the male rats. Peak values of plasma TSH in the females occurred at 9 a.m. and 7:30 p.m., when it was approximately 1.4–2.6 times greater than the mean values seen at all other times.

An analysis of variance was performed to assess the differences between the peak mean values and all other means in each sex, in both experiments. In males, the plasma TSH concentrations at 6 a.m., 3 p.m. and 4:30 p.m. were significantly greater than the means of all other times in both experiments (A: \( F = 10.30; \) df 10, 69; \( P < 0.001 \); B: \( F = 18.09; \) df 7, 48; \( P < 0.001 \)). Student's \( t \)-test revealed significant differences (\( P < 0.02 \)) between the 3 p.m. mean and the means for 4:30 p.m. and 6 a.m. No difference was observed between the 4:30 p.m. and 6 a.m. mean levels. In the female rats the plasma TSH concentration at 7:30 p.m. was similar to that at 9 a.m., and both mean values were significantly higher than all other means in both experiments (A: \( F = 5.48; \) df 10, 60, \( P < 0.05 \); B: \( F = 5.45; \) df 7, 53, \( P < 0.001 \)).

A significant difference was found between the mean plasma TSH values that occurred at 3 p.m. in both experiment in males and all the other peak means in males and females (A: \( F = 4.32; \) df 4, 30, \( P < 0.05 \); B: \( F = 4.83; \) df 4, 37, \( P < 0.02 \)). None of the other values differed significantly from each other.

Calculations were performed for all non-peak values in each sex in each experiment as well as for all non-peak values for both sexes in each experiment, in order to determine whether these values represented baseline levels and whether differences in the basal values between male and female rats could be demonstrated. No significant differences for these mean values were found between male and female rats in each experiment. No significant variations were found when mean levels of both sexes in each experiment were considered together.

Fig. 2 shows the pituitary TSH content in male and female rats. Significant fluctuations in the content of TSH in the pituitary were observed, and there was an apparent sex difference in the pattern of these changes. In the male, the lowest pituitary TSH concentrations were obtained at 6 a.m. and 3 p.m. An analysis of variance indicated that the differences between the mean content occurred at 6 a.m. and 3 p.m. in males and the means of all groups of males in Experiment A were significant (\( F = 2.86; \) df 10, 80, \( P < 0.02 \)). Similar calculations for the values of males in Experiment B revealed significant differences (\( P < 0.02 \)) between the 3 p.m. mean and the mean at all other times; however, the differences between the 6 a.m. level and all other means were not significant. In the females the lowest content of pituitary TSH was obtained at 9 p.m. An analysis of variance revealed that the value of pituitary TSH at 9 p.m. in females was significantly different from the means of all other groups of females in both experiments (A: \( F = 2.76; \) df 10, 80,
Plasma PBI in male rats during the day and night. Experimental conditions and symbols as described in the legend to Fig. 1.

\( P < 0.05; B: F = 3.47, \text{ df } 7, 49, P < 0.001 \). Similar changes were found when the data were expressed as pituitary TSH concentration.

Figs. 3 and 4 show the plasma PBI and the thyroid iodine content at different hours of the day. In males no differences were observed between the

**Fig. 3.**
The thyroid iodine content in male and female rats at different hours of the day and night. Experimental conditions and symbols as described in the legend to Fig. 1.
low mean PBI values obtained at 6 a.m., 6 p.m. and 7:30 p.m., while the
differences between each of these means and all other means values were sig-
nificant in both experiments (A: F = 3.40, df 10, 83, P < 0.05; B: F = 4.56,
df 7, 54, P < 0.01). The lowest values of plasma PBI in the female rats oc-
curred at 6 a.m., 7:30 p.m. and 9 p.m., and each of these means was signi-
ficantly lower than all other mean values (A: F = 2.73, df 10, 78, P < 0.05;
B: F = 5.12, df 6, 47, P < 0.01). No significant variations were found in thyroid
iodine content between the mean values obtained during the day and night
in each sex, or between sexes in both experiments.


discussion

Previously we found that the thyroid activity in normal male rats was in-
creased in the late afternoon and early hours of the night (Jolin & Tarín 1974;
Pallardo et al. 1976). Since thyroid function is under the control of thyro-
trophic hormone, a rise in plasma TSH concentration in the afternoon and
evening would also be expected to occur. Such a rise was, in fact, observed
in male and female rats in the present study; it occurred later in females than
in the males. The existence of a circadian rhythm for the concentration of
TSH in plasma and pituitary in rats has been suggested previously (Bakke &
Lawrence 1965; Singh et al. 1967; Retiene et al. 1968).

Our results indicate that in the rats there is a marked sex difference in the
pattern of plasma and pituitary TSH concentration under resting conditions.
We observed diurnal variations of plasma concentrations of TSH in both sexes,
but the peak levels occurred at 6 a.m. and 3 p.m. in males and at 9 a.m. and
7:30 p.m. in females. However, Singh et al. (1967) reported a single peak of
maximal TSH secretion in rats at 3 p.m. The non-peak values in each sex
showed no significant variations; and thus they can be regarded as the baseline
level. No differences in the baseline levels were found between the sexes. The
present data also show that the TSH content was highest in the pituitary gland
of male rats killed between 9 a.m. and 1:30 p.m. or between 5:30 p.m. and
3 a.m. There was a fall of TSH content in the pituitary gland of male rats
at 6 a.m. and 3 p.m.; these times correspond to the times at which the highest
levels of plasma TSH were observed. However, in female rats the pituitary
TSH content did not reflect the changes seen in the plasma TSH concentration.
Sex differences in the diurnal rhythm of TSH in the pituitary have been
reported by Retiene et al. (1968), but they did not relate their findings to the
variations in plasma TSH levels during the day and night. Little is known
regarding the cause of these sex differences in the diurnal variations of TSH.
Perhaps the gonadal hormones modify the programming of the hypothalamic-
pituitary secretion as related to recurrent events in the diurnal cycle.
The present results suggest that variations in plasma TSH are not merely a consequence of those found in circulating PBI, since in males the maximal plasma TSH levels occurred at 3 p.m. while plasma PBI are still relatively high and not while they are lower for a long period of time. On the other hand, since the rats are more active at night it is possible that the 24 h plasma TSH concentration patterns are related more closely to a higher activity at night.

REFERENCES


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