Circadian Pattern of Bioassayable and Radiommunoassayable Growth Hormone in the Pituitary of Female Rats[1] (35174)

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(Introduced by G. Pozza)

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Variations in the daily secretory activity of the pituitary gland have been extensively reported in the last few years for corticotropin (1) and the gonadotropins (2). For growth hormone (GH) a periodic secretion from the pituitary independent of blood glucose concentration or NEFA levels has been described in the human (3, 4). In the experimental animal the only findings concerning a periodicity for GH secretion are those of Bahorsky and Bernardis (5), who noticed in the rat circadian fluctuations in the pituitary content of "tibia test assayable material".

This paper reports the study on bioassayable and radioimmunoassayable pituitary growth hormone (GH) content, plasma radioimmunossayable GH, and hypothalamic growth hormone releasing (GRF) activity in female rats during a controlled 24-hr light–dark cycle.

Materials and Methods. Female Sprague-Dawley rats 22 days old were used. All animals were housed for 1 week before use in temperature-controlled, air-conditioned quarters under an artificial lighting schedule (lights on at 0600 hr, off at 2000 hr); the standard diet and water supply were allowed *ad libitum*. On the day of the experiments at each designated time, unanestethized rats (20 animals) were decapitated with a guillotine. Anterior pituitaries were removed and weighed to the nearest 0.05 mg on a microtorsion balance; adenohypophyseal tissue was then pooled by groups (20 animals/group) and homogenized in 0.9% saline. The diluted material was kept frozen until immediately before GH assay.

Stability of the frozen homogenates has been found to last as long as 6 months.

GH assay. 1. Pituitary bioassayable (BA) GH. GH activity of the samples was measured by the "tibia test" method of Greenspan *et al.* (6). Female Sprague-Dawley rats, hypophysectomized at 26–28 days of age by the transauricular approach of Falconi and Rossi (7) were obtained through the courtesy of Dr. G. Falconi. Six–8 rats were used to assay each sample. Bovine GH (1st Int. Std. WHO) was used as the reference standard. GH potencies of the pituitary homogenates were calculated by a 4-point assay according to Finney (8). Significance of differences in epiphyseal cartilage width was tested by Student's *t* test or by factorial analysis.

2. Pituitary and plasma radioimmunoassayable (RIA) GH. (a) Pituitary. For the determination of GH by RIA, samples of pituitary homogenates obtained from single glands of each of the experimental groups (6–8 glands/group) were used. Ten μl of the undiluted extract of each pituitary (previously homogenized in 0.5 ml of 0.9% saline) were used. Samples were diluted 1:1000 in phosphate buffer, 0.01 *M*, pH 7.6, and stored at —20° until assayed. The same pituitary extracts, remaining after removal of the aliquots necessary for RIA, were used for the biological determination of GH (see above).

(b) Plasma. Individual blood samples were collected from the trunk of the same animals (6–8/group) into heparinized test tubes. The plasmas were separated and frozen prior to assay for GH.

(c) GH radioimmunoassay. Pituitary and plasma growth hormone were measured by the double antibody radioimmunoassay of

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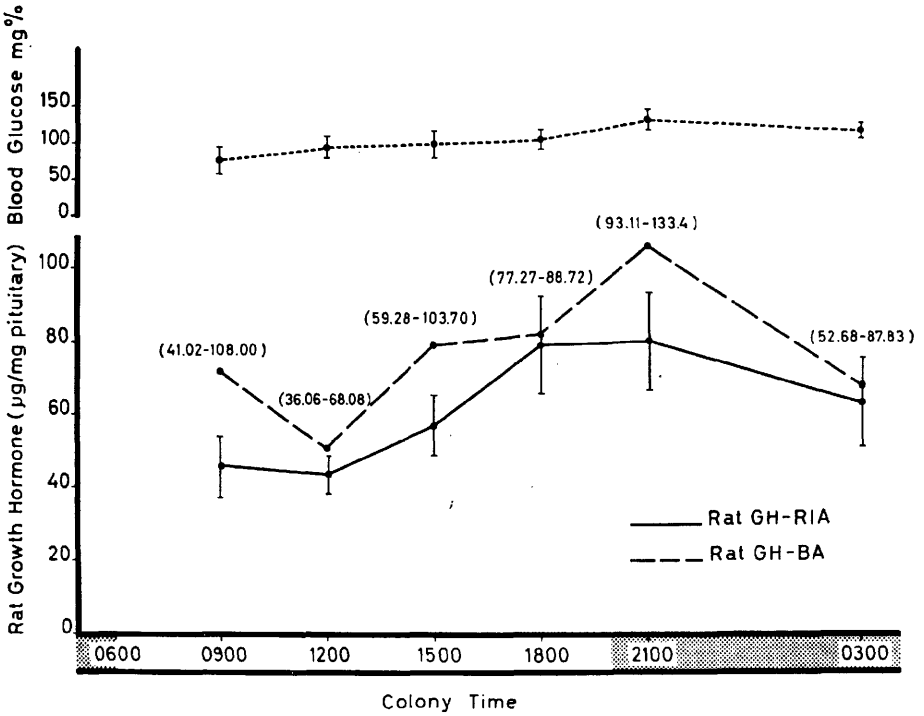

FIG. 1. Circadian rhythm of bioassayable (BA) and radioimmunoassayable (RIA) growth hormone in intact female rats. Horizontal shaded bars indicate period of darkness. Numbers in parentheses represent 95% fiducial limits for BA-GH. Vertical lines indicate standard error of the mean for RIA-GH or blood glucose.

Schalch and Reichlin (9) with minor modifications. Antibody to rat GH induced in guinea pigs was supplied by Dr. D. Schalch. Rat growth hormone was iodinated with ^{125}I (Amersham, England IMS/3), 1 mCi vial, using the method of Greenwood *et al.* (10). The specific activity obtained was in the order of 80–100 mCi/mg.

Rat GH ^{125}I before using was purified by gel filtration on Sephadex G-150 to remove the immunologically inactive components (11).

For GH standards, rat growth hormone reference preparation, NIAMD-Rat-GH-RP-1 with biological potency = 0.6 IU (bovine GH)/mg was used. Separation of bound from free hormone was obtained by precipitation of antibody-bound hormone with rabbit antiguinea pig γ-globulins. The precipitates in the tubes were collected by microfiltration through "Oxoid" membranes filter discs (2.5 cm) mounted on a Millipore microanalysis filter holder in a vacuum-filtration flask. The specificity of this assay has been well documented (9).

Test of hypothalamic GRF activity. GRF activity of stalk median eminence extracts of experimental rats was evaluated by preparing acid extracts of the stalk median eminence (SME) by the method reported previously (12). Thirty-day-old Sprague-Dawley female rats were used as recipient animals. They were given the equivalent of 2 or 1 rat SME in 0.5 ml of saline by intracarotid injection under ether anesthesia. The control recipient rats were injected with 0.5 ml of saline only. Fifteen min after injection, the recipient rats were decapitated. GH activity of their anterior pituitary gland was measured as described above. The depletion of pituitary GH in recipient animals was used as an index of GRF activity present in the extract injected.

Blood glucose. A sample of blood was obtained from experimental rats (6–8/group) at the time of sacrifice for blood glucose determinations (Biochemia Test Combination,

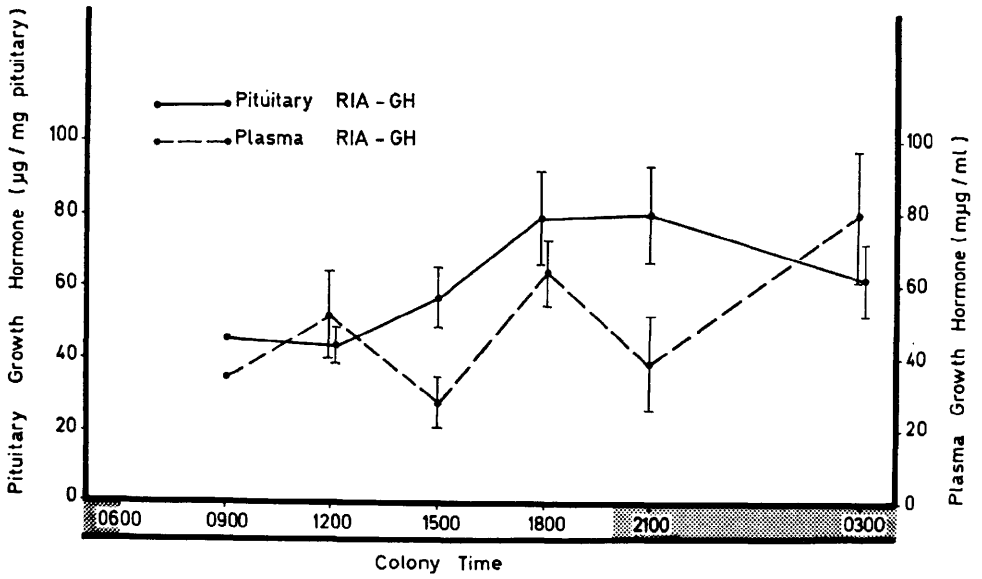

FIG. 2. Radioimmunoassayable (RIA) plasma growth hormone in intact female rats during a 24-hr light-dark cycle. The pattern of RIA pituitary GH, as represented in Fig. 1 is also depicted. Vertical lines indicate standard error of the mean.

Boehringer Mannheim GMBH).

Results. Pituitary BA-GH. The pituitary content of bioassayable growth hormone showed a rhythmic fluctuation during the 24-hr light–dark cycle (Fig. 1). The lowest GH levels in the pituitary were found at noon: 51.52 (36.06–68.08) µg GH/mg of pituitary while a progressive increase was observed during the afternoon [15 hr, 78.74 (59.28–103.70) µg GH/mg of pituitary, p NS vs 1200 hr]; 1800 hr 82.76 (77.27–88.72) µg GH/mg of pituitary, $p < 0.05$ vs 1200 hr). A peak level corresponding to 108.80 (93.11–133.40) µg GH/mg of pituitary was reached at 2100 hr ($p < 0.001$ vs 1200 hr; $p < 0.05$ vs 1800 hr) shortly after the onset of the dark period. The peak GH activity at 2100 hr was followed by a drop to 68.97 (52.68–87.83) µg GH/mg of pituitary at 0300 hr ($p < 0.01$ vs 2100 hr) with no change at 0900 hr, 72.25 (41.02–108.00) µg GH/mg of pituitary.

Pituitary RIA-GH. Pituitary content of radioimmunoassayable growth hormone showed a pattern similar to that described for bioassayable GH (Fig. 1): the lowest GH levels were found at noon (44.7 ± 8.1 µg GH/mg of pituitary) (SE) with a small enhancement at 1500 hr, 57.2 ± 8.1 µg GH/mg pituitary (p NS vs 1200 hr). Thereupon pituitary RIA-GH reached the highest levels: 1800 hr, 79.4 ± 13.5; 2100 hr, 80.0 ± 13.8 µg GH/mg of pituitary. After these peak levels, pituitary RIA growth hormone decreased, so that at 0300 hr it was 63.7 ± 11.9 µg GH/mg of pituitary and at 0900 hr it was down to 45.8 ± 8.7 µg GH/mg of pituitary. The difference ascertained between peak GH activity (1800 and 2100 hr) and levels found at 1200 hr was significant ($p < 0.05$).

Plasma RIA-GH. Wide fluctuations were noticed in the plasma RIA growth hormone during the controlled 24-hr light–dark cycle (Fig. 2). Mean GH in plasma obtained at 0900 hr was 35.4 ± 6.9 mµg/ml, a value not significantly different from that determined at 1200 hr (53.7 ± 11.9 mµg/ml).

The plasma GH concentration found at 1500 hr was 28.5 ± 7.5 mµg/ml with rapid return to preexisting values at 1800 hr (64.0 ± 8.5 mµg/ml). At 2100 the mean GH in plasma was 38.9 ± 13.3 mµg/ml which reached the peak level of 81.4 ± 20.9 mµg/ml at 0300. The differences between peak levels of GH found in plasma (1800 and 0300 hr) and lowest levels (1500 hr) were significant ($p < 0.02$).

TABLE I. Growth Hormone Releasing Activity in Stalk Median Eminence (SME) Extracts of Intact Female Rats During a 24-hr Light–Dark Cycle.

Group	Time (hr)	Material injected (no. of donor animals in parentheses)	AP equivalent assay rat total dose/4 days (mg)	Width of tibia cartilage ($\mu \pm$ SE[a])
A	—	Saline	2.0	255 ± 1.8
B	0900	2.0 SME (20)	2.0	202 ± 5.9[b]
B′	0900	1.0 SME	2.0	217 ± 4.3
C	1200	2.0 SME (20)	2.0	201 ± 2.8[b]
C′	1200	1.0 SME	2.0	218 ± 5.9
D	1500	2.0 SME (20)	2.0	205 ± 2.6[b]
D′	1500	1.0 SME	2.0	219 ± 6.0
E	1800	2.0 SME (20)	2.0	202 ± 6.2[b]
E′	1800	1.0 SME	2.0	223 ± 6.0
F	2100	2.0 SME (20)	2.0	201 ± 3.2[b]
F′	2100	1.0 SME	2.0	222 ± 6.1
G	0300	2.0 SME (20)	2.0	204 ± 2.4[b]
G′	0300	1.0 SME	2.0	219 ± 2.2

[a] Mean value of epiphyseal width of hypophysectomized rats was 156 ± 8.2.
[b] $p < 0.001$ vs saline.

Hypothalamic GRF activity. The injection of all the hypothalamic extracts induced a depletion of bioassayable pituitary GH proportional to the dose used. However, no difference was noticed in the GRF potency of the hypothalami taken at each different time-interval during the day (Table I).

Plasma glucose. Determinations of glucose in plasma of animals killed at different hours showed a gradual increase from the levels found at 0900 hr (74 ± 9 mg/100 ml) to the peak levels present at 2100 hr, (1200 hr, 93 ± 5 mg/100 ml; 1500 hr, 97 ± 8 mg/100 ml; 1800 hr, 100 ± 6 mg/100 ml; 2100 hr, 126 ± 5 mg/100 ml).

The peak concentration of plasma glucose observed at 2100 hr was followed by a slightly lower value at 0300 hr (110 ± 5 mg/100 ml).

Discussion. A significant daily periodicity in the content of pituitary growth hormone, was found in nonstressed fed female rats during a controlled 24-hr light–dark cycle.

Growth hormone progressively increased from the low levels present during the morning to a peak at 1800 and 2100 hr and then reattained the morning levels during the night.

Recently Frohman and Bernardis (13) calculated a daily secretory rate of 60–80 μg/day for RIA-GH in adult unanesthetized female rat: the difference found in the present experiments in the pituitary concentration of RIA-GH between 1200 and 2100 hr (about 35 μg/mg of pituitary) appears to be compatible with their value, taking into account the dissimilarities of experimental variables.

The daily pattern of the variation of pituitary GH content is reminiscent of that described by Bahorsky and Bernardis (5) for "tibia test assayable material" in fed female rats, but these authors observed peak levels at 1400 hr and not at 1800 or 2100 hr. The discrepancy could be explained by considering the different light–dark cycle of their colony (lights on at 0600 hr, off at 1800 hr, while our lights on was at 0600 and lights off at 2000). Onset of darkness, which coincides with the period of greatest activity in the rat (14), might be responsible for the depletion of pituitary GH apparently initiated at 1400 in their experiments and at 2100 in ours. It should be noted in this regard, that GH levels even higher than those present at 1400 hr may have been missed in the experiments

of these authors by sampling only at 6-hr intervals, rather than every 3 hr.

The similarity of results obtained in the present experiments by using bioassay or radioimmunoassay might be surprising in view of the marked discordance between BA and RIA results reported for rat GH (15–18). If one recalls, however, that BA and RIA results correlate well in measurement of growth hormone content in the unstimulated pituitary (19), the present data obtained in untreated rats are well explainable. Thus from ours and previous results (19) a pattern is emerging which indicates that "both bioassay and radioimmunoassay give approximately the same value for the concentration of GH in the pituitary of the unmanipulated rat" (16). Manipulation of the animal results in lower pituitary GH levels by bioassay than by radioimmunoassay.

Based on the assumption that the decrease of growth hormone in the pituitary is consequent to its release into the circulation, one might have predicted higher plasma GH levels during the morning than in the afternoon. This was not the case, since values to be expected were found at 1500 and 2100 hr but not at 1800 hr. The high values found in plasma at 1800 hr would imply that in the rat an increased synthesis and release of GH takes place in the late afternoon, as it has been reported recently for ACTH (20). During the night, plasma values rose considerably, even if, due to the wide range of distribution, levels found at 0300 hr, were not significantly different from those present at 0900 hr. In spite of the wide fluctuations of GH in plasma, it would appear, nevertheless, that GH peaks in plasma twice daily, at 1800 and 0300 hr.

The increase in BA and RIA pituitary GH was accompanied by an almost parallel increase in plasma glucose, and might be related to the well-known suppressive action of glucose on GH release. This possibility, however, appears to be remote considering the slight variations of blood glucose, which are not within the range necessary in the rat to decrease GH release (21). On the other hand, a periodic secretion of growth hormone apparently independent of blood glucose concentration has been reported also for the human (3, 4).

Measurement of GRF in the hypothalami of animals sacrified at different hours during the day, could not evidence excursions of GRF activity in phase with the pituitary rhythm. This fact does not necessarily imply that the diurnal fluctuations in the content of pituitary GH take place in the absence of a central nervous system involvement. Also keeping in mind that GRF content represents the net effect of synthesis and release and that this neurohormone was not measured in plasma, it would appear, nevertheless, that hypothalamic GRF remains rather stable in the rat throughout the day and does not so closely reflect the events taking place in the pituitary during a 24-hr cycle as, for instance, corticotropin-releasing factor (CRF) (20).

The existence of a circadian rhythm for GH in the pituitary calls for further investigations to define the possible endogenous or exogenous events interfering with it. Alterations or shift in the lighting regimen (D. T. Krieger, personal communication) or sleep–wake cycle (22, 23) have been recently reported to affect plasma GH in the human.

Summary. Resting levels of bioassayable (BA) and radioimmunoassayable (RIA) pituitary growth hormone (GH), radioimmunoassayable plasma GH, and hypothalamic GH-releasing (GRF) activity were determined at intervals during a controlled 24-hr light–dark cycle in intact prepubertal female rats. A gradual parallel increase in BA and RIA pituitary GH was found from late morning through afternoon, with peak levels at 1800–2100 hr. Reattainment of values close to the morning levels took place during the night.

Radioimmunoassayable plasma GH exhibited wide fluctuations with peak concentration at 1800 and 0300 hr. Measurements of hypothalamic GRF activity were unable to evidence daily excursions in phase with the clear pituitary rhythm.

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