

Ultradian Luteinizing Hormone and Testosterone Rhythms in the Adult Male Monkey, *Macaca fascicularis**

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ABSTRACT. Using the RIA for measuring serum testosterone (T) levels and the mouse interstitial cell bioassay for measuring monkey LH, we studied the secretory dynamics of these hormones in the adult male monkey. Eight sexually mature monkeys of the species *Macaca fascicularis* were sedated and blood was sampled under various protocols over 24 h. All experiments were conducted between February and June. A pronounced diurnal rhythm in the serum concentration of T was revealed by measurements from individual means summed over 4-h morning and evening intervals (0700–1100 and 1900–2300 h) in eight animals. The mean evening T levels (12.2 ± 0.8 ng/ml) were significantly greater than the morning values (9.1 ± 0.8 ng/ml; $P < 0.02$). Measurements at 20-min intervals over the 4-h periods revealed discrete pulses of T and LH secretion occurring during the morning (0700–1100 h) and evening (1900–2300 h). Analysis of T pulse frequencies and amplitudes between morning and evening

showed a significantly higher frequency of pulses occurring in the evening (1.5 ± 0.27 pulses/4 h) than in the morning ($0.25 \pm 0.27/4$ h); pulse amplitudes were not different between morning and evening samples. Samplings at 20-min intervals over 24 h showed a similar pattern. A clear relationship was found between episodic LH and T secretion during both day and nighttime sampling intervals; each individual pulse of T began within 20 min after discrete 3- to 10-fold increases in the serum LH concentration. These data show that in the male monkey, the apparent diurnal rhythm of serum T level is due to a diurnal modulation of the frequency of a pronounced ultradian rhythm in the secretion of this hormone; furthermore, these data document a nearly one to one relationship between pulsatile T and LH secretion, and suggest that the central nervous system-pituitary axis operates on a diurnal schedule to modulate the frequency of pulsatile LH secretion in this species. (*Endocrinology* 107: 1489, 1980)

A DIURNAL pattern of testosterone (T) secretion has been described for the male of human (1, 2) and nonhuman primate species (3, 4). The gonadectomized male rhesus monkey exhibits a diurnal rhythm in serum LH levels (4), but it is not known whether this is also true of intact animals. Whether normal men exhibit a diurnal rhythm in LH secretion remains controversial (5–8).

In men, both LH and T appear to be released episodically (9–12); however, the patterns of hormone levels in blood do not clearly indicate that abrupt increases in LH secretion cause corresponding increases in T secretion (13, 14). In nonhuman primates, data concerning episodic T secretion have not been reported, and the relative nonspecificity of the generally available RIA for LH (15–17) has precluded a detailed study of the secretion of this

hormone. To explore the relationship between T and LH secretion in the adult male monkey, *Macaca fascicularis*, we coupled a RIA for determining serum T levels with the mouse interstitial cell bioassay for measuring monkey LH, and we here report on the secretory dynamics of these hormones, as assessed by peripheral blood measurements over 24 h.

Materials and Methods

Monkeys

Eight sexually mature, male, crab-eating macaques, *Macaca fascicularis*, weighing 3–6 kg, were housed at a constant temperature (21 ± 2 C) in individual cages and maintained on a 12-h light, 12-h darkness cycle (lights on at 0600 h). Animals were either captured by hand or sedated with ketamine HCl (10 mg/kg, im; Parke-Davis, Detroit, MI), and blood samples were drawn from peripheral veins either through indwelling cannulae or by venipuncture. During all experimental blood sampling intervals, indoor overhead fluorescent lights were turned on to facilitate blood sampling. Blood samples were allowed to clot overnight at 6 C and then were centrifuged at $1000 \times g$ for 30 min at 4 C. The serum was separated from the cells, frozen, and stored at -20 C until assayed.

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Experimental design

All experiments were conducted between February and June. To establish the presence of a diurnal rhythm of serum T concentration and to assess the influence of general anesthesia on the T rhythm, we bled animals ($n = 8$) at 0900 and 2100 h both with and without ketamine HCl anesthesia. All subsequent bleedings were performed on animals sedated with ketamine HCl. To characterize the minute to minute dynamics of T and LH secretion, we bled animals ($n = 8$) at 20-min intervals from 0700–1100 h and from 1900–2300 h. Four additional animals were bled at 20-min intervals over the 24-h period in two separate 12-h sessions (0700–1840 and 1900–0640 h). Each bleeding interval (both 4- and 12-h sessions) was separated by at least 1 week from its corresponding day or night segment.

Hormone assays

We measured T concentration by RIA using dichloromethane extraction and purification on a Celite-ethylene glycol column with ethyl acetate in isooctane (18). Polyethylene glycol 600 was used to separate bound and free tracer. The sensitivity of the assay was 0.20 ng/ml (5 pg on the standard curve); the between-assay coefficient of variation was 8.7% and the within-assay coefficient of variation was 4.5%. Interferences from 5 α -dihydrotestosterone, 5-androsten-3 β -ol-17-one, 4-androstene-3,17-dione, and 5-androsten-3 β ,17 β -diol were less than 1%. Standards and serum samples produced parallel displacement of tritiated T. The average recovery of added steroid was $76.0 \pm 6.4\% \pm$ SD, and individual recoveries were run with each sample.

The LH bioassay was a modification of the procedures described by Van Damme *et al.* (19) and Dufau *et al.* (17). This assay is based on T production by mouse Leydig cell preparations. For each assay, two 5- to 8-week-old mice were sacrificed by cervical dislocation. The testes were removed and placed in a petri dish containing 0.3 ml aerated preincubation medium [medium 199 with Hanks' salts, L-glutamine, and 25 mM Hepes buffer; penicillin (6 mg/100 ml); streptomycin (5 mg/100 ml); 0.1% bovine serum albumin (BSA), and 10% NaHCO₃ (1.0 ml/100 ml); pH adjusted to 7.4 with NaOH]. The testes were cut with scissors into small pieces and 20 ml of the same medium were added. The cells were gently dispersed for 10–15 min with a magnetic stirrer surrounded by an ice bath; then, the medium was repeatedly drawn into and gently squeezed from a Pasteur pipette over several minutes until a homogeneous suspension was obtained. The medium was filtered through a fine nylon mesh and then preincubated for 1 h at 34 C. The cell suspension was cooled in ice water and centrifuged at $250 \times g$ for 15 min at 8 C. Sedimented cells were resuspended in 10 ml (2.5 ml/testis) incubation medium (preincubation medium plus 0.125 mM methylisobutylxanthine plus 100 IU/ml sodium heparin). The interstitial cells numbered approximately $3.0 \times 10^5/0.1$ ml medium. Incubations were performed in polyethylene tubes (13 \times 100 mm) at 34 C for 3 h; the tubes were shaken at 90 cycles/min in a Dubnoff metabolic incubator under continuous aeration of 95% O₂ and 5% CO₂. The total volume in each incubation tube was 0.22 ml. Tubes run for the standard curve each contained 0.1 ml dispersed cell medium, 0.02 ml 5% BSA in preincubation medium, and 0.1 ml incubation medium contain-

ing monkey pituitary gonadotropin (LER 1909-2; relative potency, $0.0032 \times$ NIH-LH-S1; range, 3.1–200 ng). Tubes run for the assay of serum samples each contained 0.1 ml cell medium, 0.1 ml incubation medium, serum samples at three levels (5, 10, and 20 μ l), and appropriate volumes of 5% BSA to give a constant total incubation volume of 0.22 ml. At the end of the incubation, the tubes were placed on ice, and 3.78 ml 0.01 M phosphate-buffered saline (pH 7.0) were added. Tubes were centrifuged ($1500 \times g$ for 15 min) at 8 C; the supernates were collected and stored at -20 C until assayed for T. The minimally detectable amount of LER 1909-2 was 8.3 ± 0.72 (SD) ng/incubation tube ($n = 19$); this represents the LH value read from the cell blank plus 2 times its SD. The mean interassay and intraassay coefficients of variation for pooled serum from male *Macaca fascicularis* were 13.2% and 3.9%, respectively ($n = 6$).

Data analysis

Data analysis was performed on log-normalized transformations of the raw hormonal data. Mean integrations of serum hormone levels were calculated by Simpson's rule program. For analysis of the serum T rhythm, the following definitions and criteria were applied to the observations. A pulse equals an increase of more than 3 ng/ml within 40 min; if one pulse precedes another, then the duration equals the time from baseline to baseline, where baseline equals the concentration immediately preceding a pulse. On the basis of these criteria, there were no ambiguous judgments. Mean plasma hormone concentrations, integrated serum hormone concentrations, and the T pulse frequencies, amplitudes, and durations for the day were compared with those for the night by the paired *t* test. References to either daytime (a.m.; 0700–1840 h) or nighttime (p.m.; 1900–0640) intervals represent descriptive definitions and do not delineate light-dark periods.

Results

Diurnal T rhythm

Figure 1 (*top panel*) illustrates the presence of a diurnal pattern in serum T levels calculated from individual means summed over 4-h a.m. and p.m. intervals (0700–1100 and 1900–2300 h) in eight animals. The mean p.m. T levels (12.2 ± 0.8 ng/ml) were significantly greater than the a.m. values (9.1 ± 0.8 ng/ml; $P < 0.02$). The mean serum T values measured in single samples drawn at 0900 and 2100 h without anesthesia did not differ significantly ($P > 0.2$) from those collected similarly under anesthesia.

Ultradian T and LH rhythms

Measurements at 20-min intervals over a 4-h period revealed discrete pulses of T secretion occurring during both the a.m. (0700–1100 h) and the p.m. (1900–2300 h) periods. Analysis of pulse frequency in the eight animals showed that a total of two pulses occurred in the a.m.,

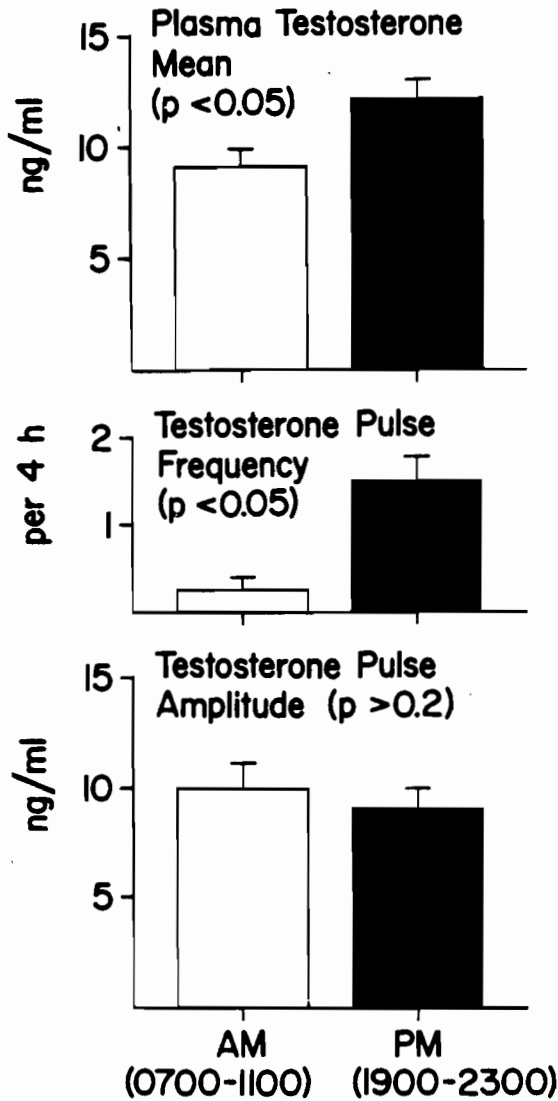


FIG. 1. Diurnal patterns of mean serum T levels, T pulse frequency, and T pulse amplitude in adult male monkeys (n = 8).

for a mean frequency of 0.25 ± 0.16 pulses/4 h; 14 such pulses occurred in the p.m., for a mean of $1.5 \pm 0.27/4$ h, (Fig. 1, middle panel). Pulse amplitude did not differ significantly between the a.m. and p.m. samples (Fig. 1, lower panel).

Blood samplings and analysis over 24 h (Fig. 2) showed a pattern similar to that found in the 4-h a.m.-p.m. sampling study. The 12-h a.m. mean T level was 4.45 ± 0.23 ng/ml, whereas the p.m. mean T level was 7.21 ± 0.46 ng/ml. Analysis of pulse frequencies again showed a greater frequency of p.m. T pulses (a.m., 0.5 ± 0.29 pulses/12 h; p.m., 3.00 ± 1.08 pulses/12 h; n = 4). Analysis of pulse amplitudes showed similar mean a.m. and p.m. peak amplitudes (9.15 ± 1.65 and 8.45 ± 1.12 ng/ml, respectively; n = 4).

Figure 3 presents six illustrations of the relationship

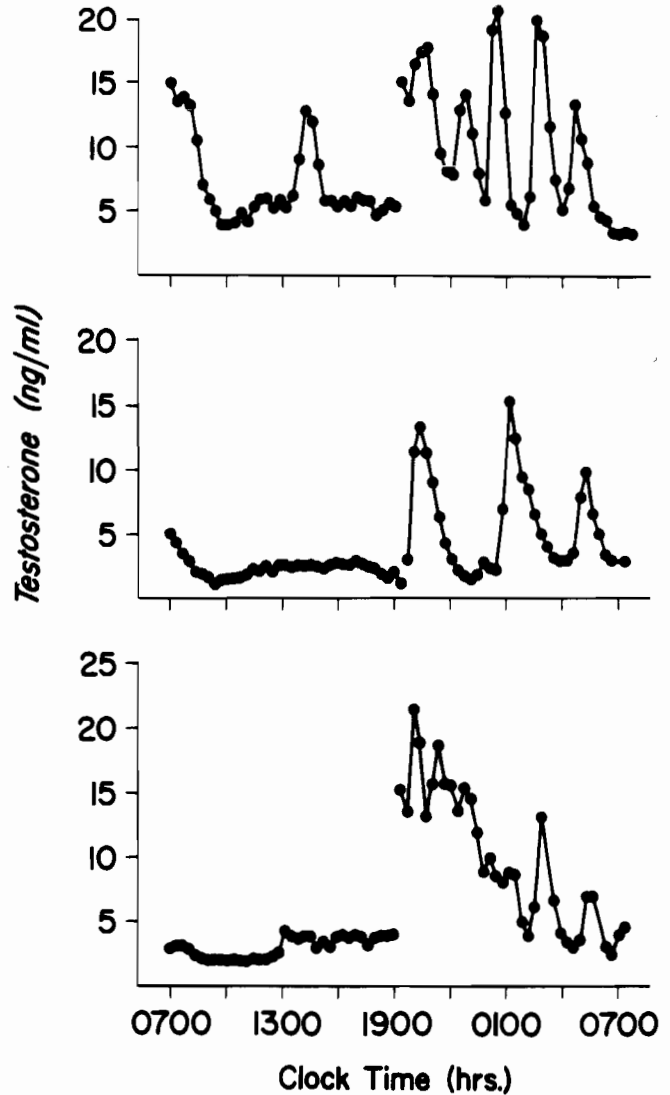


FIG. 2. Ultradian rhythm of serum T levels observed in three adult male monkeys (n = 3).

between pulsatile T and LH secretions. The three segments in the left panels are taken from a.m. sampling intervals; those on the right are from p.m. sampling. Daytime measurements revealed a very low incidence of either LH or T secretory activity, whereas p.m. measurements documented the occurrence of frequent pulsatile increases in serum levels of both LH and T. Peak LH values ranged from 1.3-7.0 $\mu\text{g/ml}$ during spontaneous surges, representing increases of up to 10-fold over basal values. Each of the discrete pulses of T was preceded by a discrete increase in the serum LH concentration. This apparent one to one relationship between LH and T existed for segments taken from both a.m. and p.m. sampling intervals. The lag time between the first recognizable increase in LH and that of T was always 20 min or less.

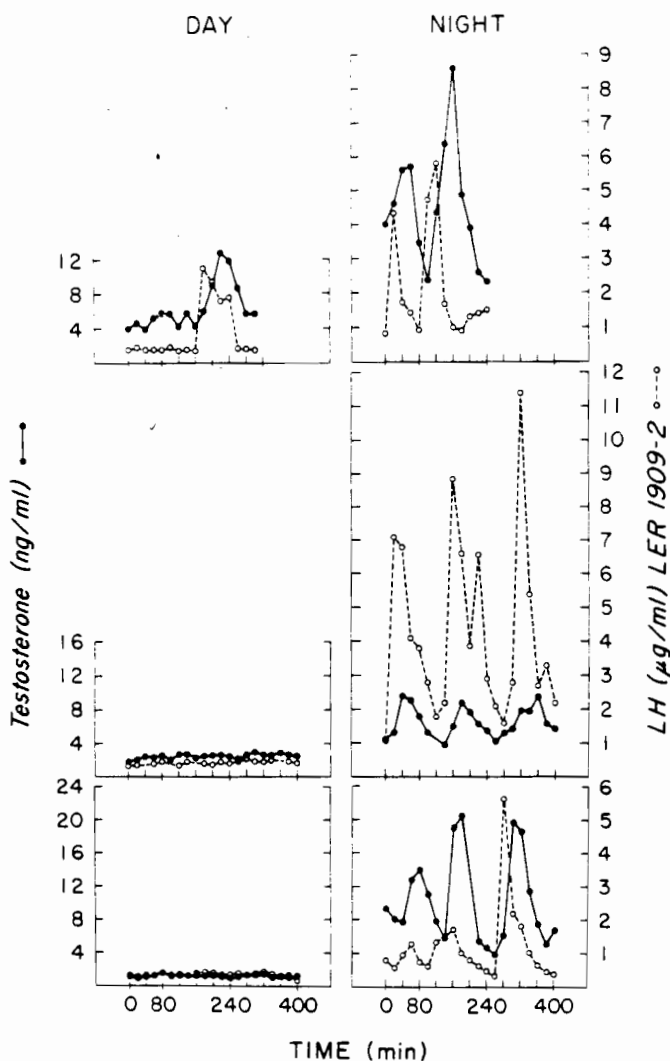


FIG. 3. Relationship between pulsatile LH and T secretion during selected segments of the a.m. and p.m. sampling intervals ($n = 3$).

Discussion

Our results demonstrate that when extended interval morning and evening blood samples are obtained from adult male crab-eating macaques, serum T levels exhibit a pronounced diurnal rhythm, with the evening segment being significantly greater than the morning. These results are similar to those reported previously for the rhesus monkey (3, 4, 20). In addition, using frequent blood sampling (20-min intervals), we have shown that an ultradian rhythm in blood T levels occurs in these animals. Indeed, the ability to demonstrate higher mean T levels at night than during the day is not due to a transition from consistently high values at night to consistently lower values during the day but, rather, to the fact that the short term episodic increases in T levels (the ultradian rhythm) are more frequent at night than in the day.

Our results also demonstrate a pronounced ultradian rhythm in blood LH levels. In all sampling intervals, both day and night, there was a virtual one to one relationship between serum LH and T activity; in every instance that measurements were performed, episodic increments in LH levels preceded T increments by 20 min or less. When similar LH increments were induced by iv infusions of LHRH (1 $\mu\text{g}/\text{kg}$) (Bremner, W. J., and R. A. Steiner, unpublished results), T increments indistinguishable from those occurring spontaneously were produced; these results are similar to those reported recently for the adult male rhesus monkey (21). The most likely explanation for these observations is that episodic secretion of T is caused by pulsatile LH release, which, in turn, is caused by the episodic production of endogenous LHRH. If this explanation is correct, we may infer that the brain of this adult male nonhuman primate operates on a diurnal schedule to modulate the frequency of endogenous pulsatile LHRH secretion and, thereby, LH and T secretion.

In the female monkey, there is no evidence that frequency modulation of LH secretion occurs either over the normal menstrual cycle or on a diurnal basis, although some evidence exists for diurnal changes in ovarian steroids (22). The lack of evidence bearing on this point may be due to the nonspecificity of the generally available RIA systems for measuring basal LH levels in nonhuman primates (15-17).

Other species in which ultradian rhythms in LH and T levels have been sought include bulls (23), rams (24), and men (13, 25). All of these species exhibit clear ultradian rhythms in LH secretion. In bulls and rams, definite episodic increments in T levels follow the LH increments (23, 24). In man, although considerable variability in T levels is found, it has been difficult in most studies to demonstrate definite increases in T after LH pulses (13), and when such increases do occur, they are very small in magnitude compared to those of other species. Similarly, when LHRH is administered to men, although the LH increments may be pronounced, it has been difficult or impossible to demonstrate increases in T levels (26-29). The reason for this relative gonadotropin resistance of the human testis is not known; the present results demonstrate that this resistance may not be characteristic of the testis of other primate species.

Many studies have investigated diurnal T rhythms in man (30). Although some investigators have been unable to demonstrate a clear diurnal rhythm, most have found that T levels are highest at approximately 0800 h and lowest at 2200 h (30). Thus, the diurnal T rhythm may be phase shifted in men compared to that in monkeys.

In men, although some evidence suggests the existence of a diurnal rhythm in LH secretion (6), most studies have been unable to document significant diurnal variations in plasma LH values (7, 8). Our observations in the

adult male monkey, demonstrating the ultradian LH and T secretory activities which appear to be physiologically linked, are reminiscent of the hormonal patterns seen in boys at the time of puberty, during which discrete nighttime bursts of LH and T secretion are observed closely linked to the sleep-wake cycle (8). Elucidation of the relationship between LH and T rhythms and the sleep-wake cycle in the monkey together with the neural mechanisms underlying these phenomena and the physiological role of these rhythms await further study.

Addendum

While this report was in press, very similar results in the adult male rhesus monkey have been presented (see Ref. 31).

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