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Regulation of Testosterone Rhythmicity by Gonadotropins in Bonnet Monkeys (Macaca radiata)

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ABSTRACT

In an attempt to study the factor(s) that regulates production of nychthemeral testosterone surges in adult male bonnet monkeys *(Macaca radiata)*, serum levels of testosterone, LH, FSH, and prolactin were monitored during a 24 h period. Only prolactin showed a significant increment in its levels coincident with that of the testosterone surge. The relationship between LH and testosterone production was studied by 1) observing the responsiveness of testes, in terms of testosterone production, to one or two injections of oLH (1 mg/injection) given 12 h apart at 0900 and 2100 h; and 2) monitoring the effect on testicular testosterone production of LH antiserum injection given at 1000, 1700, and 2100 h. That each LH injection brought about an increment in testosterone level of equal magnitude suggests that the difference in responsiveness of the testes to unchanging levels of LH at morning and night hours is not due to any alteration in substrate availability at the two time intervals. The LH antiserum experiments indicate that irrespective of the time of its administration the nocturnal surge of testosterone, which normally occurs at 2200 h, is blocked. While the antiserum prevents a rise in testosterone level, it appears not to influence basal testosterone production. The results further show that even at 2100 h, when surge testosterone production is already initiated, the testis is still highly sensitive to lack of LH, antiserum injection bringing about within 2 h a significant reduction in testosterone levels (by 69% in experimentals vs 11% in controls).

INTRODUCTION

An earlier report from this laboratory indicated that the normal nychthemeral rhythmicity in the secretion of serum testostertone (peak levels around 2200 h) seen in adult male bonnet monkeys *(Macaca radiata)* is abolished following exposure to continuous illumination (from a normal 12 h of light to a 24 h light schedule) (Mukku et al., 1976). A similar diurnal rhythmicity in serum testosterone (T) secretion has been observed in rhesus monkeys (Michael et al., 1974; Goodman et al., 1974). That the increase in serum T level in the night is due to an increased output of T from the testes and not to differential metabolic clearance or adrenal androgen secretion has also been shown (Goodman et al., 1974). Attempts to observe a significant increment in LH level correlatable to the androgen surge in the monkey have hitherto not succeeded. The present study was undertaken with a view to understanding the mechanism of regulation of nocturnal testosterone surges. The bonnet monkey *(M. radiata)* was considered a good subhuman primate model since it shows highly reproducible testosterone surges, exhibits no seasonality with respect to testosterone production (Murty et al., 1979), and repeated blood sampling, as taken in this study, has been shown not to influence gonadotropin levels.

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MATERIALS AND METHODS

Hormones and Antisera

oLH (NIH-LH-S19) when administered was given i.v. in doses of 1 mg/animal in saline. Antiserum (a/s)

used here was raised in bonnet monkeys to LH (NIH-LH-S19), and characterized for freedom from non-specific antibodies and crossreactivity with monkey LH (Prahalada et al., 1975). It was injected i.v. in doses of 10 ml/animal over a 1–2 min period. The time of injection varied depending upon the experimental protocol. Control animals received an equivalent dose of normal monkey serum.

Animals and General Methodology

Monkeys were all adult male bonnets weighing 7–8 kg each. Details of monkey husbandry used in this study were essentially similar to that described by us (Mukku et al., 1976). Blood was withdrawn (3 ml at a time) from the femoral vein of unanesthetized monkeys housed in squeezeback cages. This permitted withdrawal of blood from each monkey at consecutive time periods. During night blood sampling, particular care was taken not to expose the animals to direct light. In keeping with the practice in this laboratory of recycling monkeys from one experiment to another after sufficient rest in between, monkeys used for establishing hormonal patterns (Exp. 1) were also used as controls in Exp. 2 and 3. The "experimentals" were recruited from the stock after establishing that they, too, exhibited normal testosterone nychthemeral rhythms.

The radioimmunoassay system used for measuring LH, FSH, and testosterone were essentially the same as described by Rama Sharma et al. (1978) and Mukku et al. (1976). Prolactin (PRL) levels in serum were measured by using the human prolactin radioimmunoassay kit supplied by NIAMDD Bethesda, MD. The cross reactivity of the PRL RIA system with other pituitary hormones like growth hormone, thyrotropin, and gonadotropins is negligible (Dr. A. F. Parlow, personal communication). The results were analyzed for statistical significance using Student's t test, and analysis of variance on untransformed data whereever applicable.

RESULTS

Measurement of Testosterone, LH, PRL, and FSH over a 24 h Period (Fig. 1)

Testosterone levels showed a clear diurnal rhythmicity, the low and high levels occurring at 1000 and 2200 h, respectively (2.82 ± 0.92 ng/ml vs 21.34 ± 4.14 ng/ml; $P<0.001$). The levels started rising at 1800 h and returned to basal values by 0600 h. Of the three pituitary trophic hormones measured, LH, FSH, and PRL, only PRL showed a significant increase ($P<0.001$) in its level between 1800–0400 h of the day. PRL increased from a basal level of 115 ± 9 ng/ml at 1000 h to 287 ± 32 ng/ml at 1800 h. Further, the peak levels of testosterone and prolactin observed at 2200 h exhibited a correlation coefficient of $r = +0.94$. The radioimmunoassay system used for monitoring LH, FSH, and testosterone levels were validated earlier to measure these hormones in the bonnet monkey serum (Rama Sharma et al., 1978; Mukku et al., 1976). The ability of the hPRL radioimmunoassay system to measure bonnet monkey PRL is shown by the parallel inhibition curve that monkey serum exhibited in this assay (Fig. 2).

Assessment of the Ability of Testes to Respond to Exogenous LH Given at Different Times of the Day (Fig. 3)

Within 2 h of LH injection (0900 h), serum testosterone levels significantly increased (from a basal value of 2.68 ± 1.63 ng/ml to 21.41 ± 4.62 ng/ml; $P<0.025$); this heightened level was maintained for 2–4 h. These LH-treated monkeys, however, failed to exhibit normal nocturnal T surges. Control monkeys did not show any response to the vehicle, but exhibited normal nychthemeral T surges (Fig. 3A). Administration of 1 mg of LH at 0800 h and 2100 h of the same day to another group of monkeys resulted, by 2 h of each injection, in an increase in serum T level. The magnitude of increment in both instances was similar (Fig. 3B).

Effect of Neutralization of LH by Passive Immunization with Ovine LH Antiserum at Different Times of the Day on the Diurnal Testosterone Secretory Pattern (Fig. 4)

While injection of LH a/s at 1000 h resulted in the abolition of the T surge for that night alone, administration of LH a/s at 1700 h or 2100 h resulted in the abolition of the nocturnal T surges of that day and the following day. The acute effects of LH neutralization on T levels are seen in Table 1. The antibody, in addition to preventing a rise in T over basal levels (e.g., when given at 1000 or 1700 h), brings about a sharp fall in T level when given close to the normal T surge (at 2100 h).

DISCUSSION

Although attempts to find correlative changes in serum LH levels with testosterone nocturnal surges exhibited by the adult male bonnet monkey did not meet with success, the present study clearly demonstrates that production of testosterone in surge amounts is an LH-controlled phenomenon. Of the three gonadotropic hormones, LH, FSH, and prolactin, only prolactin showed a significant change in its serum level coincident with testosterone

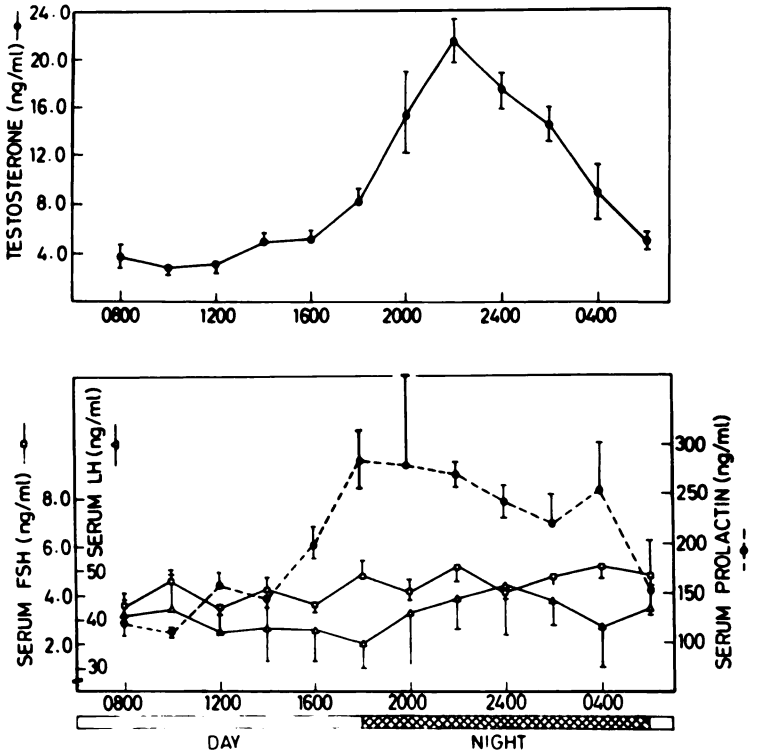

FIG. 1. Serum levels of FSH, LH, PRL, and testosterone over a 24 h period in adult male bonnet monkeys *(M. radiata)* △——△, LH; ●——●, testosterone; □——□, FSH; and ●----●, prolactin. n = 5. For details of assay see text.

surge. Studies with other animals have shown that LH and testosterone levels exhibit a typical cause-and-effect relationship, there being coincident rises in the level of the two hormones (Racey et al., 1975; Berndtson and Desjardins, 1974). The absence of such relationship in the bonnet monkey is puzzling. It is, however, conceivable that the increment in LH during night hours is of smaller magnitude, and the sensitivity of the radioimmunoassay system used here may be the limiting factor in discerning it. Although Goodman et al. (1974) have reported that in the rhesus monkey no correlative change between LH level and T surge is observed, recent reports from the same laboratory using more sensitive techniques claim to have detected such changes (Plant, 1979). The situation with regard to the human is also not very clear since there is no unanimity of opinion as to observing correlative changes in LH level concomitant with T surge (Judd et al., 1974).

The possibility that basal levels of LH itself could be regulating production of nocturnal testosterone surges cannot be overlooked. Thus, responsiveness of the testis to LH stimulation could vary depending upon the time taken to

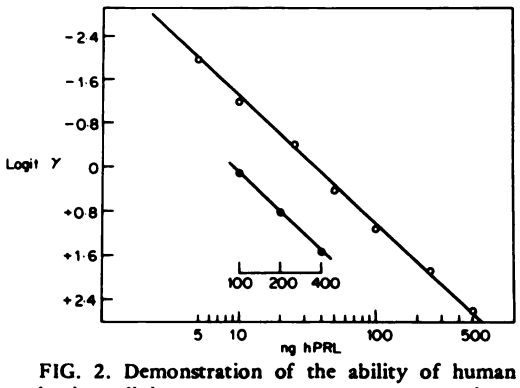

FIG. 2. Demonstration of the ability of human prolactin radioimmunoassay system to measure bonnet monkey serum prolactin. The radioimmunoassay was conducted using the NIAMDD hPRL RIA kit. The assay protocol was essentially similar to that standardized for pituitary gonadotropins (Rama Sharma et al., 1978). ○——○, hPRL standard; ●——●, monkey serum (μl).

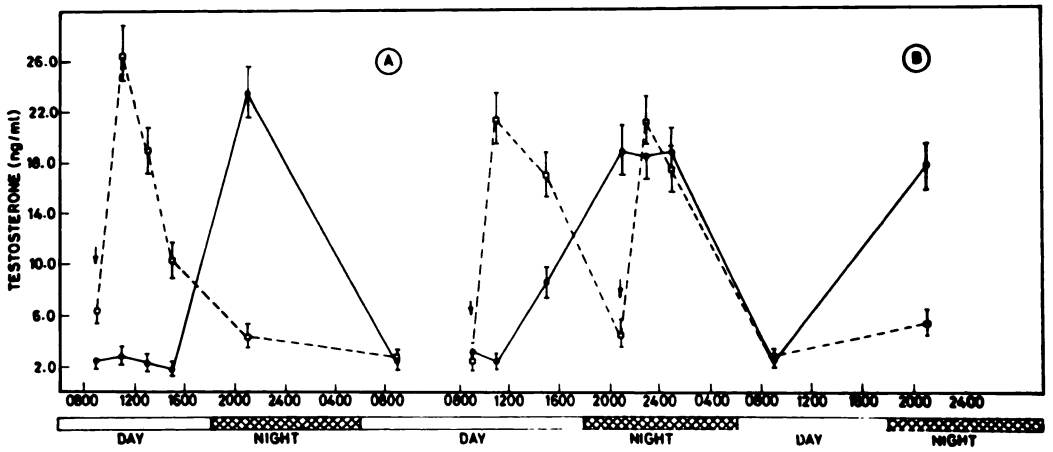

FIG. 3. Effect of LH administration at two time intervals of the same day (0900 and 2100 h) on serum testosterone levels. Controls (n = 5) and experimentals (n = 3) in each group. □----□, Experimentals; ●——●, controls; ↓ indicates time of LH injection i.v. (1 mg/injection)

restore substrate stores depleted by production of testosterone surge and this time could be as long as 24 h. This has been checked by measuring response to a bolus of LH (1 mg dose) given in the morning as well as in the evening of the same day. Each injection brought about an acute and significant rise in testosterone level (from a basal value of around 3 ng/ml to 21

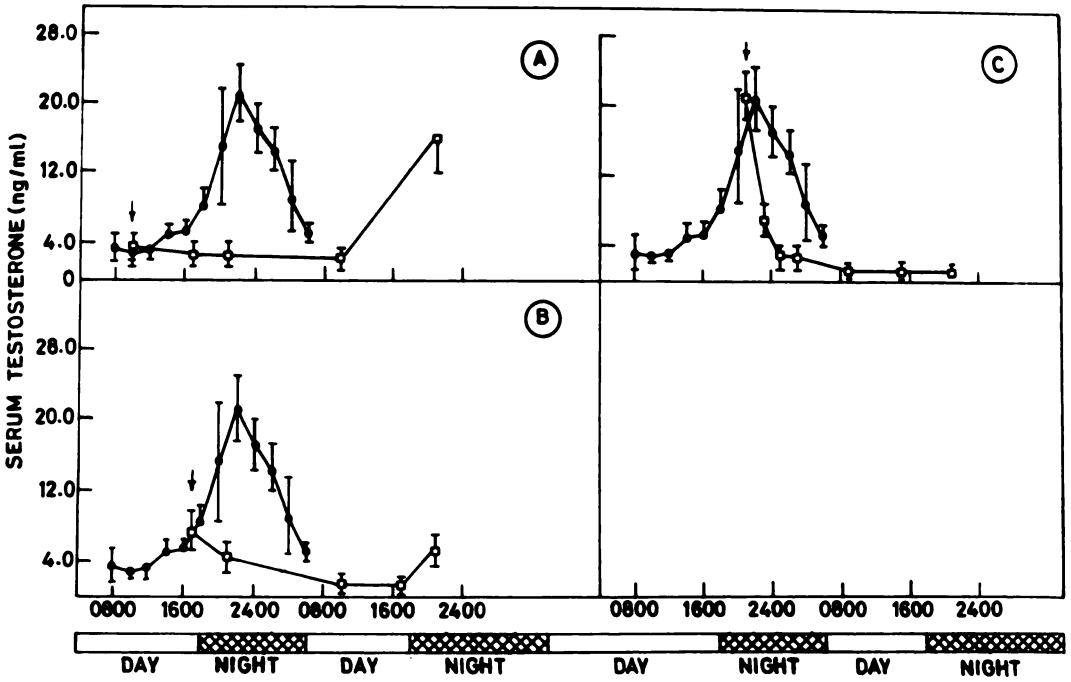

FIG. 4. Effect of LH antiserum injection at different times of the day on serum testosterone levels. 10 ml of LH a/s injected i.v. at 1000, 1700, and 2100 h to three groups of monkeys. Control group received 10 ml of normal monkey serum (NMS). In each of the experimental groups n = 4, and in control groups n = 3. ●——●, Controls; □——□, experimentals; ↓ indicates the time of a/s injection.

TABLE 1. Acute effects of LH antiserum injection on testicular testosterone production.

Group (Time of injection)	Testosterone levels at different times[a] (ng ± SD/ml serum)			
	0	2 h	4 h	6 h
Group I (2100 h)				
C[b]	20.58 ± 4.5	18.26 ± 4.3 (11%)[c]	12.46 ± 4.3 (44%)	8.73 ± 2.9* (57%)
E[b]	21.66 ± 3.7	6.60 ± 2.2**,† (69%)	3.06 ± 1.8**,† (86%)	2.90 ± 1.7**,† (86%)
Group II (1700 h)				
C	6.43 ± 2.5	...	19.76 ± 4.4*** (207%)	...
E	7.20 ± 2.6	...	4.60 ± 2.1††	...
Group III (1000 h)				
C	3.88 ± 1.9	...	...	6.21 ± 2.5 (60%)
E	3.48 ± 1.8	...	...	3.21 ± 1.8

[a]The reduction of T levels observed over appropriate time controls is as follows: 1) at 2, 4, and 6 h of Group I (2100 h): 64%, 74%, and 67%, respectively; 2) at 4 h of Group II (1700 h): 77%, and 3) at 6 h of Group III (1000 h): 48%.

[b]10 ml NMS or LH a/s was given i.v. at different times indicated; C = received NMS, and E = received LH a/s. Note that normal T surge (21.34 ± 4.1 ng/ml) occurs at 2200 ± 1 h.

[c]Numbers in parentheses refer to percent of change over 0 h control.

*P<0.05, **P<0.01, ***P<0.025; significantly different from 0 h controls.

†P<0.05, ††P<0.01; significantly different from the appropriate time controls.

ng/ml; P<0.05), the amount produced each time equaling that normally seen as nocturnal surge. This showed that testicular responsiveness to LH does not necessarily change because of large amounts of T produced 12 h earlier. Following 1 or 2 LH injections, the immediate nocturnal T surge does not occur and it takes 2–3 days for the natural rhythm to become reestablished. The reason for this, although presently unclear, is that it could be related to change in sensitivity brought about by partial desensitization. This is currently being investigated.

Administration of LH a/s at any time of the day inhibits nocturnal T surges showing clearly that this is an LH-dependent phenomenon. The recurrence of the natural T surge is essentially dependent upon the a/s half-life and occurs within 2–3 days of a/s injection. Considering that serum LH levels do not show appreciable change during the 24 h period and that the volume and titer of the a/s given at the three different times are the same, the degree of neutralization of LH achieved by giving a/s at 1000, 1700, or 2100 h would be expected to be equivalent. An examination of serum T levels at short times after a/s injection shows that while the antibody is able to inhibit increase in production of T over the basal value it has no effect on basal T production itself. The possibility that this T is coming from the adrenal, however, cannot be excluded. The first two time periods chosen (1000 h to 1600 h, and 1700 to 2100 h) represent the rising phase in T level, it being increased during the period by 60% and 200%, respectively. LH a/s injection effectively prevents this increment. Since T surge occurs in these animals normally at 2200 ± 1 h, one could expect that at least by 2100 h, the time of the third injection, the testes would be fully programed to produce T in surge amounts and to be minimally influenced by changes in serum LH levels. The results, however, show that a/s injection even at this late hour is totally effective in blocking T production, suggesting that the testes must be highly sensitive to changes in levels of circulating LH at this time.

An increase in testicular sensitivity to unchanging levels of circulating LH can perhaps be brought about by increasing tissue receptor concentration. There is considerable circum-

stantial evidence to suggest that both PRL and/or FSH could increase LH receptor concentration (for review see Bartke et al., 1978). The role of FSH in this process, at least in monkey, is doubtful, since passive immunization of adult male bonnet monkeys with a potent FSH antiserum over a prolonged period of 120 days did not lead to a reduction in either serum or testicular T levels (Murty et al., 1979). Whether the increased level in prolactin seen in the evening hours in the present study is of any physiological significance as far as testosterone production in the monkey is concerned remains to be investigated.

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