Response of the Hypophyseal–Testicular Axis in Monkeys to Stalk–Median Eminence Extract[1] (36755)

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(Introduced by C. H. Sawyer)

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The secretion of testosterone by the testis is regulated by luteininizing hormone (LH). An elevation in serum LH after the administration of crude (1–4), purified (5–8), or synthetic (9) LH-RH[3] in rat and man indicates that LH in the male, as in the female, is released from the adenohypophysis in response to a neural hormone.

Although LH and testosterone (T) are secreted in the adult male in a more steady state than similar gonadotropin and ovarian steroid patterns in the female, evidence in rabbits and bulls suggests that stimuli such as coitus elevate T and hence LH.

In preliminary studies with rhesus males we were unable to detect significant changes in circulating levels of LH within 1 hr of coitus. Hence we were curious about the responsiveness of the male pituitary gland to hypothalamic neural hormones which govern gonadotropin release and the pattern of T secretion in relation to circulating LH changes. The lack of available purified or synthetic LH-RH at the time the studies were initiated, plus our knowledge of the response of the female rhesus monkey at different stages of the menstrual cycle to an infusion of ovine stalk–median eminence extract (SMEE), prompted us to initiate these studies in the male rhesus monkey.

Specifically, we were interested in comparing the response of LH and T secretion after an injection or a systemic infusion of SMEE of ovine origin into adult male monkeys and in studying the subsequent circulating patterns of these two hormones.

Materials and Methods. Five adult male rhesus monkeys (*Macaca mulatta*) ranging from 7.6 to 9.6 kg were used. All were clinically normal, healthy monkeys on a diet of Purina monkey chow and fresh fruit kept singly in well-protected, semioutdoor cages. The preparation of ovine SMEE and of cerebral cortex extract (CCE) has been described (10). LH was measured by radioimmunoassay (11). Purified ovine LH (LER-1056C2) was used for radioiodination with ^{125}I. The antiovine LH serum (GDN-15), diluted 1:40,000 with 1:400 normal rabbit serum and phosphate buffered saline, was used to measure LH in duplicate samples of 200 μl of serum. The standard was a partially purified preparation obtained from rhesus pituitaries (LER-M-907D) with an LH biological activity (OAAD) of 0.025 NIH-LH-S-1 units/mg (12). Concentrations of LH in serum from an ovariectomized monkey averaged 975 $\pm$ 24 ng/ml (LER-M-907D) in 9 different assays. Concentrations of T were determined with a competitive protein binding assay (13) previously described (14). The within-assay coefficient of variation for concentrations less than 1 ng/ml was 13.3%; average percentage recovery, 67.8; for concentrations greater than 4 ng/ml, 7.8%; average percentage recovery, 60.6 $\pm$ 6.8 SD. The between assay coefficient of variation for concentrations greater than 4 ng/ml was 15.2%; average percentage recovery, 50.2 $\pm$ 6.0 SD. The water blank, *i.e.*, the lower limit of sensitivity

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[3] LH-RH denotes an abbreviation for luteinizing hormone releasing potency and is interchangeable with the term LRF found in several references. It is assumed that the SMEE used in this study contains the natural chemical responsible for LH release.

as well as an element in precision, was 41 ± 46 SD pg/ml in 15 different runs; average percentage recovery, 51.7 ± 11.4 SD. This blank was not subtracted. Some of the later samples were assayed by a modification (15) of the original method, in which blanks and corrections for recovery are eliminated by carrying the standards through all procedures.

All SMEE and CCE infusions were done through an indwelling cannula in the femoral vein between 9:00 AM and 2:00 PM with sedation by Innovar and Nembutal. Two males were injected with 2 dosages of SMEE [1/12 and 1/3 hypothalamic equivalent (Heq) wet wt] at 40- to 90-min intervals. Two-milliliter blood samples were taken beginning with a preanesthesia sample and then at 8- to 10-min intervals up to about 3 hr. In later experiments 1-ml blood samples were taken beginning with a preanesthesia sample and followed by a 20-min base line period consisting of samples at 0-, 10-, and 20-min postanesthesia; a 45-min CCE infusion period with sampling beginning at 5 min after infusion was begun and continued every 10 min; a 120-min SMEE infusion period during which samples were taken at 5 min after the infusion was begun, then at 15, 20, 60, 90, and 120 min; and a postinfusion period of 1 hr during which samples were taken every 30 min. Two infusions were carried out with 2 Heq (wet wt) of CCE and 2 of SMEE, 2 with 2 Heq of CCE and 1 of SMEE, and 2 with 1 Heq of CCE and 2 of SMEE.

Results. Anesthesia caused a decline of mean serum LH levels in 5 males from 0.70 to 0.43 μg/ml within 15 to 20 min. In male No. 1867, serum LH increased from 50 ng to 19 μg/ml within 5 min after 1/3 Heq SMEE but increased to only 2.6 μg/ml after 1/12 Heq SMEE. The response of T showed fluctuating peaks and reached 9.4 ng/ml after the high dose (Fig. 1). In another male (not shown in Fig. 1), LH increased in response to 1/3 Heq SMEE from less than 1 to 13 μg/ml in 5 min and fell promptly. After an injection of 1/12 Heq SMEE, LH rose only to 1.5 μg/ml. T responded in fluctuating peaks reaching 5.2 ng/ml.

During continuous infusions, LH remained

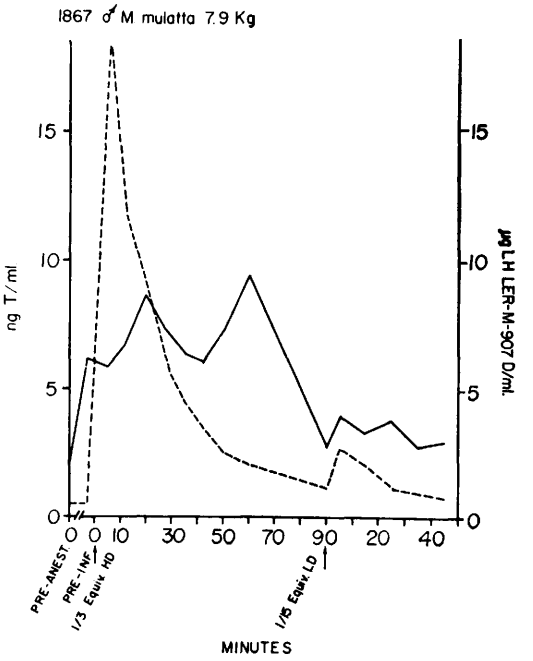

FIG. 1. Concentrations of plasma LH (- - -) and plasma T (—) following the infusion of SMEE by iv injection (↑). LH concentration is expressed in terms of a partially purified extract of rhesus monkey adenohypophysis (LER-M-907D) with a biological activity of 0.025 × NIH-LH-S-1 by OAAD assay. The dosages refer to equivalences of hypothalamic wet weight.

at levels near 1 μg/ml throughout the base line and CCE infusion periods (Fig. 2). The LH rise in response to SMEE was always apparent by 5 min. Maximum LH (2.4 to 16.7 μg/ml LER-M-907D) was reached between 60 and 90 min after the beginning of SMEE infusion and began to fall to the base line within 30 min after SMEE was stopped. Testosterone, on the other hand, began at more variable levels from not detectable to 21.4 ng/ml. In the 4 experiments in which T levels initially were high, T fell throughout the base line and CCE infusion periods. In one monkey, a T peak, apparently unrelated to any LH rise, occurred during the CCE infusion period. Unlike LH, T did not always rise within 5 min of the beginning of SMEE infusion but T maximum (2.1 to 9.9 ng/ml) was reached at 15 to 90 min after initiation of SMEE. A nonparametric procedure, the

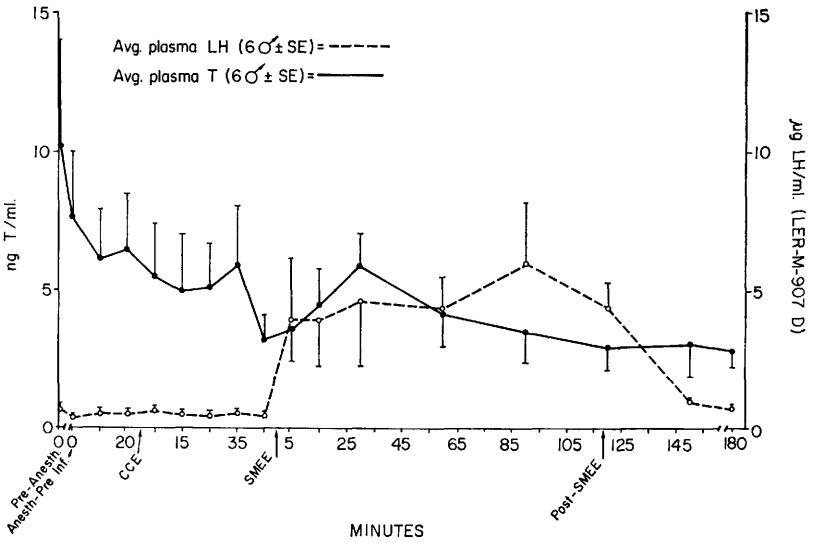

FIG. 2. Concentrations of plasma LH (- - -) and plasma T (—) followed SMEE infusion in 6 male rhesus monkeys. Means and standard errors, see Fig. 1 legend for other details.

sign test (16), yielded a significance level of 3% (2-sided) or 1.5% (1-sided) for both T and LH for the increase when the 45-min CCE sample and the maximum SMEE sample of each animal were compared. In contrast, a comparison of the 5-min CCE sample with the 45-min CCE sample showed either a slight drop or a nonsignificant rise in LH in all 6 experiments.

Similarly, in all 6 experiments a comparison of the 5- and 45-min CCE samples showed either a drop or a nonsignificant rise in plasma T concentration. However, unlike LH, either the 45-min CCE sample or the 5-min SMEE sample may represent the nadir of T concentration that precedes the rise during SMEE infusion. Moreover, T, unlike LH, did not remain elevated throughout the SMEE infusion (Fig. 2).

Discussion. These experiments indicate that the iv administration of ovine SMEE, either as an injection or as a continuous infusion into male rhesus monkeys, elicits a rapid release of LH followed by a somewhat slower and less predictable rise in circulating T presumably from the pituitary and testis, respectively. Nonspecific effects of SMEE at sites other than the pituitary have previously been discussed in a study with rhesus females and discounted (10). In that study SMEE injected on different days of the menstrual cycle resulted in marked elevations in serum LH within 5 min which returned to base line by 30 to 90 min after the injection. The response to SMEE was similar in both the follicular (day 5) and luteal (day 21) phases of the cycle. The response of males to SMEE infusion was similar to that of females, *i.e.*, serum levels increased, remained elevated during infusion, and promptly fell to baseline when the infusion ceased. For further comparison, the injection of 1 Heq of SMEE into mature, intact male rats produced a rise of about 1.5-fold; longer times were not investigated (1). In 3 children, the infusion of 17.5 Heq/kg of ovine SMEE resulted in a 2- to 10-fold increase in plasma LH concentration within 5 min, followed by a fall to the base line by 120 min (3).

There are few studies in which the release of T after LH can be compared. After the injection of 500 IU HCG into male rabbits, plasma T concentration was increased 25-fold in 30 min. After copulation or precoital excitation plasma T concentration increased 6- and 4-fold, respectively. Plasma LH was not measured (17). In an *in vitro* study of decapsulated rat testis, various LH preparations added to the media resulted within 30 min in almost 2-fold increases (18). In a study of

the effects of ejaculation in the bull (19) and rat (20), no significant effects on LH levels could be demonstrated within 30- and 20-min postejaculation, respectively. However, in another study of the effects of sexual stimulation on plasma concentrations of LH and T in the bull, Katongole, Naftolin and Short (21) found pronounced cyclical fluctuations in LH and T over 24 hr. Each LH peak was associated with a T peak but there was, as in the present study, no apparent correlation between the height of the LH peaks and that of the T peaks. LH concentrations ranged from 5 to 50 ng/ml and T ranged from 2 to 20 ng/ml over a 24-hr period. Sexual stimulation (sight of a cow, teasing, or ejaculation) caused an immediate rise in plasma LH (25- to 30-fold). Only if the T level was low at the time was the LH peak followed by a T peak. When T levels were already high at the time of LH release the testis seemed to be incapable of further response. If a rise in T occurred, it was apparent by 30 min after the LH response and amounted to about a 3-fold increase. The 24-hr T levels in the bull are strikingly similar in shape though not in degree to the T peaks in our 2 experiments involving pulse infusions. Other points of similarity are the low T response given by our monkeys after high pre-SMEE infusion levels and the timing of the T response which generally began 10 min after the LH rise. The striking fluctuations in plasma LH over a 24-hr period in both bulls and rams (22) were not seen in our monkeys during the comparatively short period (1.25 to 1.5 hr) before SMEE infusion. However, the period of the LH peaks reported was generally 2 to 4 hr. Similar rhythmic oscillations in plasma LH with a period of about 1 hr have been reported in ovariectomized female monkeys (23); intact females, however, showed low and relatively unchanging levels of LH measured at 10-, 20-, and 30-min intervals over a 6-hr period in either the follicular or the luteal phase of the cycle. Thus, the base line levels of LH in these intact females closely resemble those in our intact males. Furthermore, serum LH concentrations in men measured hourly for 24 hr were without periodic peaks (24).

During CCE infusion plasma LH concentrations tended to remain unchanged. However, in 4 out of 6 experiments, plasma T concentrations fell throughout the baseline and CCE infusion periods. The reason for these high initial T concentrations is not known. It is unlikely that the stress of capture is responsible because ACTH (25) and cortisol (26) are negatively correlated to plasma T, nor is their effect likely to be seen over a short interval (27).

These early high levels do not seem to have exerted any inhibitory effect on the response of plasma LH to SMEE. Also, in bulls high T levels did not seem to inhibit the LH release that followed sexual stimulation (21).

Obviously, the dynamic interaction of LH and T in the male monkey requires longer periods of sampling and the testing of other factors before all the interrelationships can be defined.

Summary. After an injection or an infusion of an extract from ovine stalk–median eminence (SMEE) into the femoral vein of mature rhesus males, peripheral serum levels of luteinizing hormone (LH) and testosterone (T) were measured by radioimmunossay and competitive protein binding, respectively. Within 2 to 3 min the SMEE injection initiated a 2- to 30-fold increase in serum LH which rapidly returned to base line whereas an SMEE infusion lasting 120 min resulted in a prompt elevation of serum LH which persisted throughout the infusion period. Infusions of cerebral cortical tissue extract (CCE) caused no change in the base line values of LH (430 to 550 ng/ml LER-M-907D) although anesthesia appeared to lower levels by 10 to 25%. Preanesthesia concentrations of T were high (>12 ng/ml) in 4 of 8 trials, decreased more slowly than LH after anesthesia and CCE infusions, and began to rise later than LH, reaching a maximum of 2- to 12-fold above base line within 15 to 90 min after the beginning of SMEE administration. Although circulating levels of both hormones appeared to increase after SMEE, time-sequence relationships could not be established because of variability in the magnitude and duration of maximum response.

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