Patterns of Luteinizing Hormone in Serum Following Administration of Stalk-Median Eminence Extracts to Rhesus Monkeys[1] (36416)

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(Introduced by C. H. Sawyer)

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Acid extracts of stalk-median eminence (SMEE) and purified porcine LH–RH[2] induce ovulation in estrous rabbits (1–3) and in "pentobarbitone-blocked" proestrous rats (4–7). These hypophysiotropic preparations elicit similar patterns (8, 9) of release of luteinizing hormone (LH) in ovariectomized rats treated with estrogen (E) and progesterone (P). In intact animals the magnitude of LH response following a specified injection of LH–RH is influenced by circulating levels of E and P (3, 10). Although the peripheral levels of E and P during the menstrual cycle of women (11, 12) and monkeys (13–15) are qualitatively similar to levels found during the cycle of subprimate species, the time course of diminished progesterone is, by comparison, clearly prolonged during the first half of the menstrual cycle. During this 10–14-day interval circulating levels of LH appear relatively stable (16–18) until the preovulatory surge. This LH surge accompanies rising levels of E (13) and can be elicited early by injections of E (19) or can be blocked by injections of P (18). Such data clearly indicate a gonadal–pituitary relationship, however, the responsiveness of the pituitary to hypophysiotropic stimulation at various stages of the menstrual cycle is unknown. This study reports circulating LH changes in intact rhesus females following injections or infusions of SMEE at different stages of the menstrual cycle and following injections of progesterone or an antiestrogen, U-11, 100A.[3] A portion of these results have been reported in abstract form (20).

Methods. Sexually mature female monkeys (Macaca mulatta), weighing 4.2–7.1 kg, which had exhibited three or more consecutive menstrual cycles of 25–32 days were utilized in this study. Menstrual bleeding was monitored by daily insertion of a cotton-tipped applicator stick into the vagina. Day 1 of a cycle was designated as the first day of menses.

Extracts of ovine stalk-median eminence tissue (SMEE) were injected or infused into monkeys under tranquilized sedation (Innovar, 1 mg/kg) via a cannula in the femoral vein at various stages of the menstrual cycle and on day 12, 13, or 14 of the cycle following 6–8 days of steroid injections. Two nonsedated monkeys were also tested. Blood samples were obtained from the femoral vein prior to sedation, after sedation and at 5–30 min intervals following SMEE administration. Concentrations of luteinizing hormone (LH) in 100–200 μl of serum were determined by radioimmunoassay (21). LH concentrations are expressed in terms of a partially purified preparation from rhesus pituitaries (LER-M-907D) with a biological activity (OAAD) of 0.025 NIH-LH-S-1 units/mg.

Ovine hypothalamic tissues were received as frozen blocks (PelFreez, Rogers, AR).

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[2] In this manuscript, LH–RH denotes an abbreviation for luteinizing hormone-releasing potency and is interchangeable with LRF as found in several references.

[3] U-11, 100A = 1-{2-[P-(3,4-dihydro-6-methoxy-2-phenyl-1-naphthyl) phenoxy] ethyl}-pyrrolidine hydrochloride. The compound was a gift of the Upjohn Company, Kalamazoo, MI.

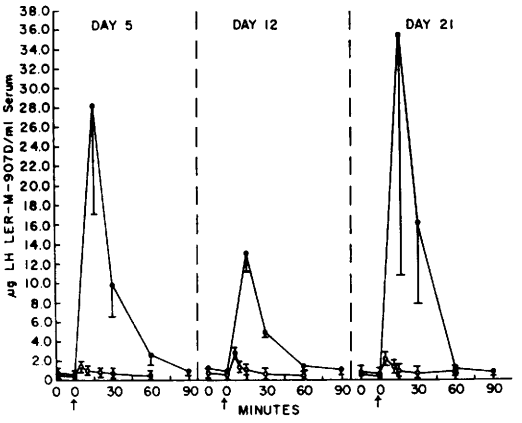

FIG. 1. Average concentrations of LH in the serum of four rhesus females measured at 15–30-min intervals following an injection (time denoted by ↑) of 1 eq of SMEE at day 5, 12, or 21 of their menstrual cycles (solid circles) or an equal wet w/v quantity of diencephalic tissue extract (open circles). Units of LH, ± SE (vertical lines below points), are expressed in terms of a partially purified extract of rhesus monkey adenohypophysis with a biological activity by OAAD = 0.025 × NIH-LH-S1 (LER-M-907D).

The median eminence region was dissected from surrounding tissues and was homogenized and extracted in cold 0.1 *N* HCl (1 SMEE/ml), frozen in small ampules and neutralized with 1.0 *N* NaOH prior to administration as described previously (9). Control procedures included: (a) determination of LH contamination of the SMEE preparations by radioimmunoassay, (b) absorption of SMEE extracts with an antibody against ovine LH and (c) acidic extraction of equivalent amounts of both occipital cortex, and diencephalic tissue located rostral to the median eminence and dorsal to the optic chiasm and injection as described for the SMEE.

In trial 1, sequential changes in serum LH concentration were measured in four females following iv injection of one equivalent SMEE on day 5, day 12, and day 22 of their menstrual cycles. Ovaries of each female were examined by laparoscopy three days after each SMEE injection to assess follicular and luteal morphology. As controls, the same females received an equivalent dosage by weight of diencephalic tissue extract on each of the appropriate days in a subsequent cycle.

Trial 2 involved a total of seven monkeys. The response to three or four different doses of SMEE was compared in individual monkeys during the early follicular (days 5–7), ovulatory (days 9–13) and midluteal (days 19–23), phases of the cycle. Two different SMEE extracts were tested and since their LH-releasing potencies did not differ, the data were pooled between extracts within stage of the menstrual cycle. As a control, extracts of cerebral cortex equivalent to or greater than the SMEE wet tissue w/v or "LH-absorbed" SMEE, were injected into four and three animals, respectively.

In trial 3, 12 females were divided into three equal groups of 4 monkeys. Females of group 1 were injected with 0.5 mg of progesterone sc daily on days 6–12 of the cycle. Individuals of group 2 were administered 0.5 mg of an antiestrogen, U-11,100A,[3] sc on days 7–12. These treatment regimens have both been demonstrated to block the midcycle rise in serum LH which normally precedes ovulation (22). The third group of females were not injected and served as controls. On day 12 of the cycle each female was sedated (1 mg/kg Innovar im) and injected iv with three doses of the same SMEE preparation at approximately 2-hr intervals. Blood samples from the femoral vein were collected at 5–15-min intervals for LH assay. Laparoscopic examination of the ovaries on day 14 was conducted in two progesterone-treated and two antiestrogen-treated monkeys.

In trial 4, three monkeys were injected with progesterone as in trial 3 and infused on day 12 via a femoral cannula for a 5-hr interval with approximately two equivalents of SMEE (.005 SMEE/min). LH concentrations were monitored at 10–30-min intervals. On day 14 the ovaries of each animal were examined by laparoscopy for evidence of recent ovulation.

Results. Serum concentrations of luteinizing hormone (LH) in intact monkeys were markedly elevated from a baseline of 0.5 μg (LER-M-907D)/ml to 28.0 ± 10.3, 12.0 ± 1.8 and 35.0 ± 24.0 μg/ml on Days 5, 12, and 21 of the cycle, respectively, by 5 min

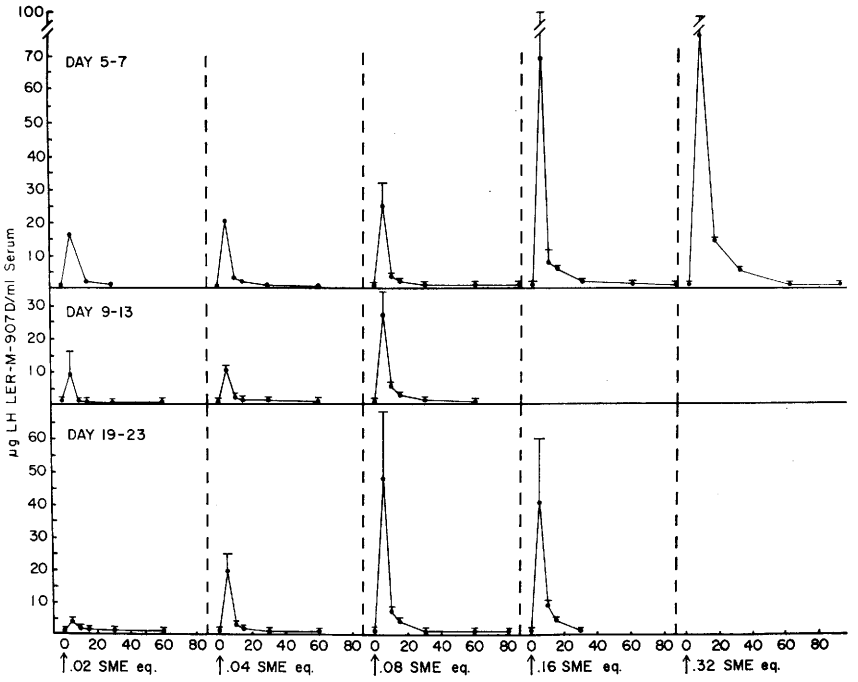

FIG. 2. Serum LH concentrations following iv injection (↑) of .02–.32 equivalents (eq.) SMEE at 2-hr intervals on days 5–7, 9–13, and 19–23 of the cycle. The numbers of monkeys at each stage of the cycle were 6, 4 and 5, respectively. Standard error of means are shown by vertical lines above each point. Refer to Fig. 1 legend for other details of chart.

after the iv injection of 1 equivalent (eq) of SMEE (Fig. 1). The rate of LH decline in serum was similar at each of the three stages of the cycle and had dropped to baseline levels within 90 min after injection. The magnitude of response to SMEE did not differ significantly ($p > .05$) during the three stages of the cycle. Injections of extract from diencephalic brain, rostral to the median eminence, did not cause appreciable increase in serum LH (Fig. 1). Examination of the ovaries of each monkey two days following SMEE revealed that ovulation did not occur as a result of the elevated LH pulse since no recent corpora lutea were present on day 7 or 23. Two monkeys had new corpora lutea at day 14 and two females possessed a large 8–9-mm follicle.

When dosage of SMEE was reduced from 1 eq to lower levels (.02–.32 eq), the rise in serum LH was also decreased and tended to be dose related (Fig. 2). The response to different doses of SMEE at each of the three stages of the cycle was similar except that .02 eq of SMEE prompted less of an LH pulse during the luteal phase (days 19–23) of the cycle than occurred on days 5–7 or 9–13 (Fig. 2). SMEE, absorbed with an anti-ovine LH antibody, injected into three monkeys on day 5 of the cycle resulted in a comparable rise in serum LH as was noted with similar dosages of unabsorbed SMEE. Extracts of cerebral cortex produced no elevation in serum LH in four monkeys. The length of the menstrual cycle was not affected by injection of SMEE (pretreatment *vs.* SMEE cycles; 28.9 ± 1.3 *vs.* 29.1 ± 2.5 days, $p > .05$).

The response to different doses of SMEE was similar in monkeys following seven daily injections of 0.5 mg of progesterone prior to injection of SMEE on day 12 and in untreated monkeys (Fig. 3). On the other hand, injection of 0.5 mg of an antiestrogen[3] daily for 6 days preceding SMEE challenge reduced the rise in serum levels of LH by 50–400% when compared with the levels of

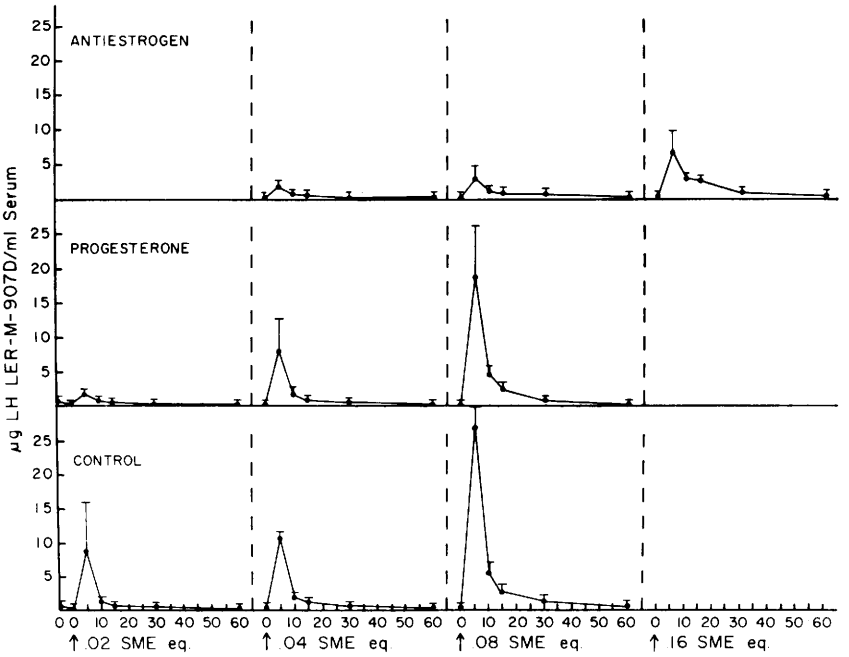

FIG. 3. Average concentrations of LH in serum (4 monkeys/group) following iv injections of .02–.16 eq SMEE at 2-hr intervals into females (a) pretreated with a sc injection of 0.5 mg of an antiestrogen, U-11,100A, on Days 7–12 (b) preinjected with 0.5 mg of progesterone, sc, on Days 6–12 (c) untreated with steroidal agents. The SMEE were administered on Day 12 of the cycle in all cases. See Fig. 1 legend for other details.

LH following comparable dosages of SMEE injected into nonsteroid- or progesterone-treated monkeys (Fig. 3). Following SMEE, ovaries of monkeys examined by laparoscopy showed no evidence of recently formed corpora lutea. Menstrual cycles were shortened significantly by both progesterone (14.8 ± 0.8 days) and antiestrogen (21.2 ± 2.0 days) injections as compared to nonsteroid-treated controls (28.2 ± 1.9 days).

Infusions of SMEE, in contrast to rapid injection, elicited LH patterns that differed markedly (Fig. 4 *vs.* 3). During a 5-hr infusion of SMEE, serum concentrations of LH increased rapidly, remained elevated until the termination of the SMEE and then declined to near baseline within 60–90 min. After injection of progesterone on Days 6–12, the infusion of SMEE for 5-6.5 hr into three monkeys produced similar patterns in serum LH typical of the one exemplified in Fig. 4. On day 14, 2 of the 3 monkeys possessed a recently formed corpus luteum suggestive of ovulation.

Discussion. These studies demonstrate that injections or chronic infusions of stalk-median eminence extract (SMEE) into rhesus monkeys can produce an increase in serum luteinizing hormone (LH) by induction of the release of LH from the pituitary. In all experiments the SMEE was administered iv; therefore, we can not exclude the possibility of some indirect action of the SMEE at sites other than the adenohypophyseal cells which produce LH. LH contamination of the SMEE is recognized (23, 24), however, it is unlikely that this factor could account for the elevated levels of serum LH we report. The radioimmunoassayable LH in the SMEE could account for less than 3% of the levels of LH in serum following SMEE injections and SMEE absorbed with an anti-ovine LH antibody elicited serum LH changes equivalent to unabsorbed SMEE.

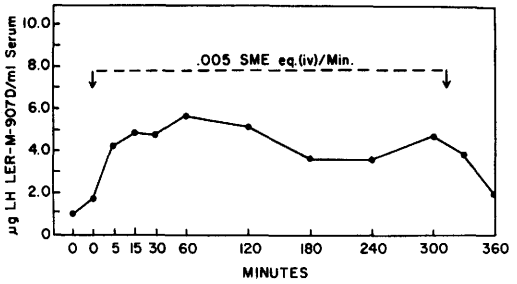

FIG. 4. Pattern of serum LH in a monkey injected with 0.5 mg of progesterone sc on days 6–12 and infused continuously for 5 hr on Day 12. LH concentration in the serum increased within 5 min after start of the infusion and returned to baseline within 60–70 min after SMEE cessation.

The possibility that the SMEE stimulated release of endogenous LH–RH can not be excluded; however, injections of extract from cerebral cortex or diencephalan, rostral to the median eminence, produced no appreciable change in serum LH. The possibility that the elevation measured in serum LH following SMEE is due to alterations in metabolism or inactivation of circulating LH has been considered elsewhere (9) and rejected. The slight LH rise following diencephalic extract may be due to the LH contamination in this preparation or to LH–RH found in this area of the brain (25).

Our results clearly indicate that the pituitary of the rhesus monkey is not refractory to SMEE at any period of the menstrual cycle tested. The responsiveness as determined by the quantity of LH released following increasing dosages of SMEE was similar during follicular, midcycle and luteal stages of the cycle or following injections of progesterone. The regimen of exogenous progesterone used will block the spontaneous preovulatory rise in serum LH (18, 22). The failure of progesterone to alter the pattern of LH elicited by SMEE suggests its inhibitory influence on the spontaneous midcycle surge of LH from the pituitary is indirect, as we have previously hypothesized (22). Ovulation did not accompany the increased levels of LH elicited by SMEE injection. Although the lack of follicles in a preovulatory state may have contributed to an absence of ovulation on cycle days 5–7 or 19–23, failure to sustain elevated levels of serum LH may also have been a factor. Chronic *infusion* of SMEE for 5–6 hr in "progesterone-blocked" monkeys maintained elevated levels of serum LH and ovulation accompanied this response in two of three monkeys, whereas a similar steroid treatment plus *injection* of SMEE failed to elicit ovulation although large follicles were present in the ovaries of these monkeys. Evidence in other species indicate circulating levels of LH must be sustained for several hr to insure normal ovulation (25–27). Yamaji *et al.* (19) have recently demonstrated that an elevation of circulating estrogen levels for at least 12 hr will induce a discharge of LH equal in magnitude and duration to the spontaneous midcycle surge. An association of increased estrogen titers preceding the preovulatory LH surge during the normal menstrual cycle have been noted also (12, 13, 15).

In the present study, injection of an antiestrogen, U-11,100A,[3] for 6 days prior to injection of varying dosages of SMEE greatly reduced the magnitude of elevation in serum LH. This refractoriness to SMEE stimulation indicates estrogen is a more important hormone than progesterone in feedback regulation of LH in the monkey.

If the action of SMEE in prompting LH release was direct, the evidence indicates a portion of the stimulatory action of estrogen is on the pituitary cells which secrete LH. Whereas this interpretation is consistent with that of evidence in other species (3, 10), the role of progesterone in regulation of LH appears to differ in the monkey from that in other species. Certainly the difference in refractoriness between antiestrogen- and progesterone-treated animals suggests different sites of action for these agents in the monkey.

Summary. Concentrations of luteinizing hormone (LH) were determined in serum samples collected from the femoral vein of rhesus monkeys at 5–15-min-intervals following administration of ovine stalk-median eminence extract (SMEE). Following iv injections of .02–.32 eq SMEE, a dose-related response was observed in serum LH. A marked increase in LH of 3–50 × baseline occurred

within 5 min of SMEE injection. This rise in serum LH returned to baseline within 60–90 min. Chronic iv infusion of SMEE for 5–6.5 hr elicited an immediate rise in serum LH and a plateau which lasted until the infusion ceased. The increased concentrations of serum LH prompted by equivalent dosages of SMEE given at Days 5–7, 9–13, or 19–23 of the menstrual cycle or following injection of 0.5 mg of progesterone sc on days 6–12 were comparable. Antiestrogen, U-11, 100A injected sc at 0.5 mg daily on days 7–12, significantly reduced the LH released by SMEE injections. The decreased response to SMEE stimulus following antiestrogen, but not progesterone injections, suggest these compounds influence LH release via different mechanisms. In control, progesterone- and antiestrogen-treated monkeys the iv injection of SMEE did not induce ovulation, whereas two of three "progesterone-blocked" females did ovulate following a 5–6.5 hr chronic infusion indicating the duration of LH elevation was critical.

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