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Ovarian Influence on Gonadotropin and Prolactin Release in Mated Rabbits

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In 17β-estradiol (E)-treated ovariectomized (OVX) rabbits, the coitus-induced luteinizing hormone (LH) surge is only one fourth that in ovarian-intact rabbits. In this study, we determined the pattern of the coitus-induced gonadotropin release, i.e., LH and follicle-stimulating hormone (FSH), in OVX + E animals without or with continuous 3-wk treatment of 20-α-hydroxypregn-4-en-3-one (20αP). For positive and negative experimental controls, ovarian-intact rabbits were either mated or sham mated, respectively. The pituitary hormones prolactin (PRL) and growth hormone (GH) were measured to serve as collateral controls for gonadotropins. The addition of continuous 20αP in OVX + E does fail to stimulate a coitus-induced LH surge equal in magnitude and duration to the LH surge in ovarian-intact rabbits. Postcoital levels of FSH were greater in OVX + E + 20αP animals than those in OVX + E rabbits. Coitus induced a PRL surge in ovarian-intact and OVX + steroid-treated females, but not in mated males, thereby suggesting a gender difference in this neuroendocrine circuit. Neither coitus nor steroids altered plasma GH values in female or male animals. We conclude that chronic administration of neither E nor E + 20αP can restore full-scale gonadotropin surges in OVX rabbits, whereas replacement of one or both of these steroids is sufficient for a coitus-induced PRL surge. Moreover, the presented observation that activin stimulates hypothalamic gonadotropin-releasing hormone (GnRH) release suggests a possible involvement of ovarian proteins in the production of a full-scale coitus-induced GnRH/LH surge.

Key Words: Gonadotropins; prolactin; growth hormone; 20α-hydroxypregn-4-en-3-one; activin; coitus.

Introduction

In a reflex ovulating species such as the rabbit, coitus triggers the hypothalamic secretion of gonadotropin-releasing hormone (GnRH), which then activates the preovulatory luteinizing hormone (LH) surge from the pituitary (for review, *see* refs. *1* and *2*). The magnitude of this LH surge can range from 30- to >300-fold above precoital levels in female New Zealand White rabbits with intact ovaries *(3,4)*. Previous findings in our (unpublished data) and other laboratories *(5)*, however, have shown a greatly diminished coitus-induced LH surge in ovariectomized (OVX) rabbits treated with estrogens, either 17β-estradiol (E) or estradiol benzoate, which encompasses a range of only 2- to 10-fold above precoital levels. These data are consistent with the hypothesis *(5)* that ovarian factors other than estrogens play an integral role in facilitating a full-scale LH surge in the rabbit (i.e., a coitus-induced LH surge equal in magnitude and duration to the LH surge in ovarian-intact rabbits).

One possible ovarian factor is the steroid 20α-hydroxypregn-4-en-3-one (20αP), secreted in abundance by intact female rabbits at various reproductive stages *(6–9)*. Coitus induces a major enhanced secretion of ovarian 20αP *(5)*. Based on this observation and the findings that intraovarian venous infusion of varying doses of LH stimulates 20αP secretion and that 20αP infusion into OVX, E-treated rabbits enhanced the magnitude of LH secretion similar to levels in ovarian-intact rabbits, Hilliard et al. *(5)* suggested a positive feedback function for 20αP on the postcoital surge of LH. However, a subsequent study *(6)* with a more direct LH assay during intravenously infused 20αP failed to show an enhancement in the magnitude of the coitus-induced LH surge, thereby casting doubt for a role of 20αP in the facilitation of a coitus-induced LH surge.

An E-filled Silastic capsule implanted subcutaneously into an OVX rabbit will allow sexual performance, but coitus with a male fails to stimulate a full-scale postcoital LH surge. This unpublished finding motivated us to reexamine the role of 20αP. We reasoned that continuous

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exposure of the hypothalamohypophyseal axis to both E and 20αP, which occurs in ovarian-intact rabbits *(8,9)*, but was not examined in the earlier experimental models *(5,6)*, may be a critical requirement for maintaining complete neuroendocrine sensitivity to coital stimulation. The present study was initiated to test this hypothesis.

Results

Plasma Levels of E and 20αP

The E and 20αP implants released expected amounts of the steroids into the circulation. Plasma levels of E before mating were similar ($p > 0.05$) among the ovarian-intact (9.0 ± 0.84 pg/mL), OVX + E (9.0 ± 1.42 pg/mL), and OVX + E + 20αP (9.29 ± 1.87 pg/mL) animals. Plasma 20αP levels in ovarian-intact rabbits (1.63 ± 0.56 ng/mL) were also similar ($p > 0.05$) to those in OVX + E + 20αP females (1.36 ± 0.31 ng/mL).

Plasma Levels of LH

Figure 1 A–D presents the mean LH surge patterns before and during the 4 h after coitus for each of the four treatment groups. The mated intact does (Fig. 1B) exhibited a well-defined surge, beginning immediately following mating, peaking near 90 min at levels >30 ng above basal values, and lasting for over 180 min after coitus. By contrast, OVX + E (Fig. 1C) rabbits exhibited a small but significant ($p < 0.05$), increase in LH within 1 h after mating, although both the amplitude and timing of the LH increase were markedly reduced compared with the LH surge in ovarian-intact females. The addition of 20αP in the OVX + E animals (Fig. 1D) did not induce an LH surge resembling the one in ovarian-intact rabbits (Fig. 1B). Figure 1E presents a comparison of LH levels among the four groups at each time point. There was no difference between the OVX + E and OVX + E + 20αP animals at any time during the mating trial. Mean LH levels in ovarian-intact rabbits were higher ($p < 0.05$) than mean LH levels in either the OVX + E or the OVX + E + 20αP animals between 60 and 210 min after mating and in sham-mated animals between 30 and 240 min after coitus.

Plasma Levels of Follicle-Stimulating Hormone

A brief but definitive coitus-induced follicle-stimulating hormone (FSH) surge was observed only in ovarian-intact rabbits (Fig. 2B). Although postcoital FSH levels in some OVX + E and OVX + E + 20αP animals appeared to be higher than the respective precoital level, the group averages were not significantly different ($p > 0.05$) (Fig. 2C,D). Furthermore, FSH levels in OVX + E and OVX + E + 20αP rabbits were two- to four-fold higher than FSH levels in ovarian-intact rabbits both before and after coitus (Fig. 2E). The addition of 20αP in OVX + E animals further increased ($p < 0.05$) blood FSH levels at 15 and 30 min after coitus (Fig. 2E).

Plasma Levels of Prolactin and Growth Hormone

Mating induced a remarkable increase in prolactin (PRL) secretion in both ovarian-intact and OVX, steroid-treated females that lasted approx 60 min (Fig. 3A–D). The amplitude and pattern of the PRL increase after coitus were similar ($p > 0.05$) among ovarian-intact, OVX + E, and OVX + E + 20αP rabbits (Fig. 3E). Moreover, none of the five mated males exhibited any increase in PRL after coitus (Fig. 4A). Consequently, postcoital levels of PRL were higher ($p < 0.05$) in females than in males (Fig. 4B).

In contrast with PRL, blood growth hormone (GH) levels did not increase ($p > 0.05$) after coitus in either females (Fig. 5A–D) or males (Fig. 4C). Furthermore, there were no differences ($p > 0.05$) in GH levels at any time point during the experiment among the four groups of females (Fig. 5E) or between males and females.

Push-Pull Perfusate GnRH

Infusion of activin into the arcuate–median eminence increased the concentration of GnRH in push-pull perfusate (Fig. 6). The mean value of GnRH during the activin infusion (4.7 ± 1.8 pg/mL) was higher ($p < 0.05$) than the mean GnRH value prior to activin infusion (0.9 ± 0.6 pg/mL). Moreover, activin appeared to increase only the amplitude of a GnRH pulse without altering the frequency of GnRH pulses. The increase in GnRH persisted for as long as the activin infusion (i.e., 3 h).

Discussion

These results confirm those of two previous reports in rabbits *(5,6)* indicating that ovarian factors are required for a full-scale discharge of pituitary LH in response to coitus. Either acute injections of E *(10)* or continuously administered E (current study) can restore coital behavior, including lordosis for male mounting and ejaculation. Furthermore, the addition of physiological amounts of 20αP to exogenous E does not alter coital behavior in OVX rabbits. However, neither continuous exposure to E nor E + 20αP before, during, and immediately following coitus was sufficient to fully sensitize the hypothalamic-pituitary axis to coital stimulation in that the mating-induced LH surge was much smaller in both OVX + E and OVX + E + 20αP females than in ovarian-intact rabbits (Fig. 1). Hilliard et al. *(7)* observed that rabbit ovaries not only secrete 20αP during the estrous stage, but also produce a massive surge of 20αP that persists throughout the period of the mating-induced LH surge. Moreover, when a "simulated 20αP surge" was given intravenously into OVX + E females, the magnitude of the coitus-induced bioactive (i.e., enhanced 20αP secretion) LH surge was greatly enhanced *(5)*. These observations led to the hypothesis that in ovarian-intact female rabbits, coitus initially induces an increase in LH discharge from the pituitary, which stimulates ovarian pro-

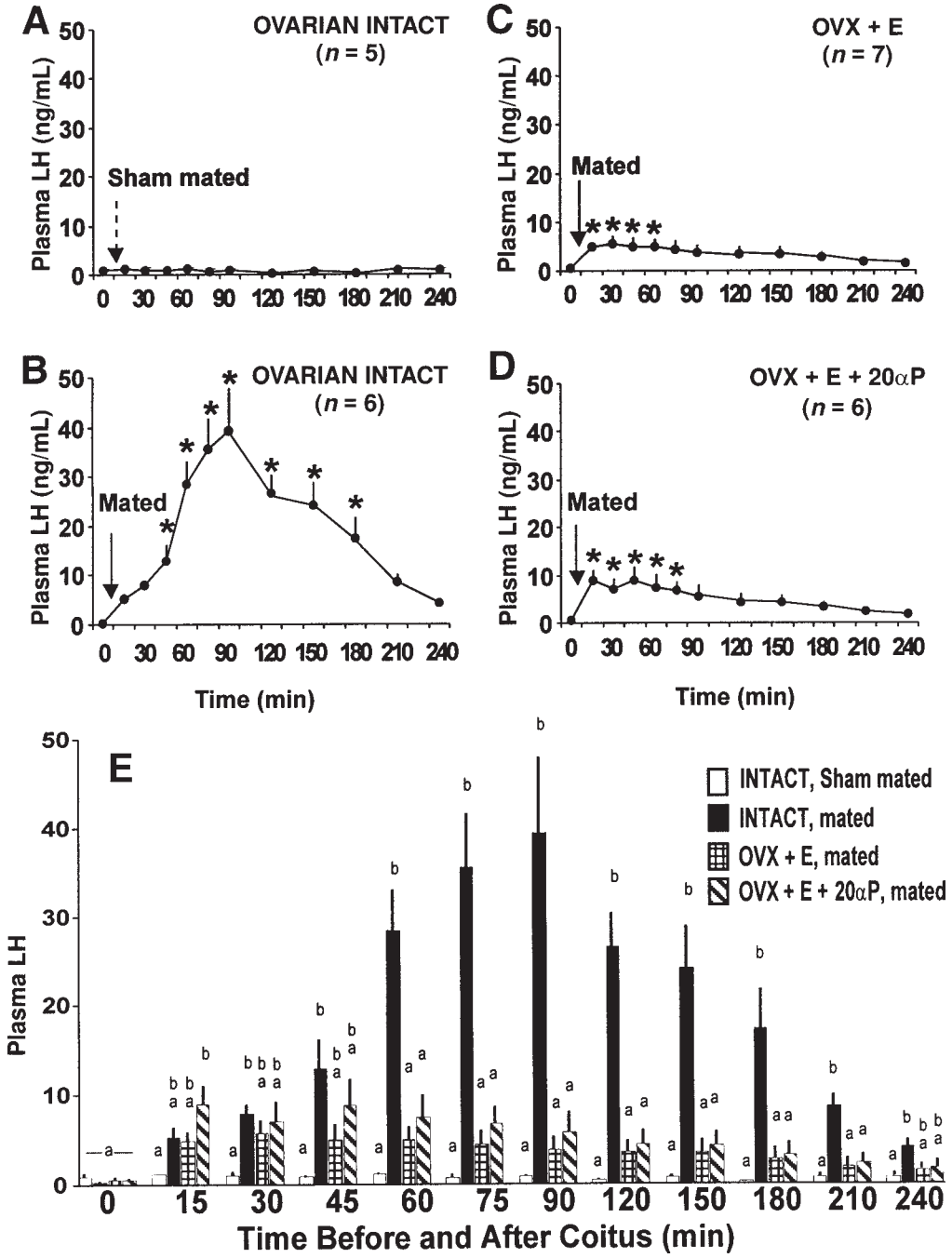

Fig. 1. Mating-induced LH release in ovarian-intact and OVX rabbits treated with 17β-estradiol (OVX + E) or E and 20α-hydroxyprogesterone (OVX + E + 20αP). **(A–D)** Asterisks indicate significant ($p < 0.05$) difference in values compared to the 0 time (precoital) value. **(E)** Different letters above two bar graphs within each time point indicate a difference ($p < 0.05$) between the two means.

duction of 20αP that feeds back on the hypothalamic-pituitary axis to stimulate and maintain a full-scale LH surge *(5,11)*. However, later studies using an LH radioimmunoassay (RIA) and iv infusion of 20αP either within 15 min after coitus *(6)* or immediately after GnRH infusion *(12)* failed to show an enhancement in the magnitude of the LH surge in OVX rabbits, thereby casting doubt that 20αP has a role in the coitus-induced LH surge.

Although this conundrum remains unresolved, several studies have demonstrated that 20αP can facilitate GnRH/LH secretion. For example, 20αP can induce an LH and FSH surge in E-treated OVX rats *(13)*. In women primed with E, 20αP (but not 17αP) administered during the midfollicular phase of the menstrual cycle induces an LH surge *(14)*. Moreover, pulsatile administration of 20αP has been shown to stimulate the release of GnRH from the

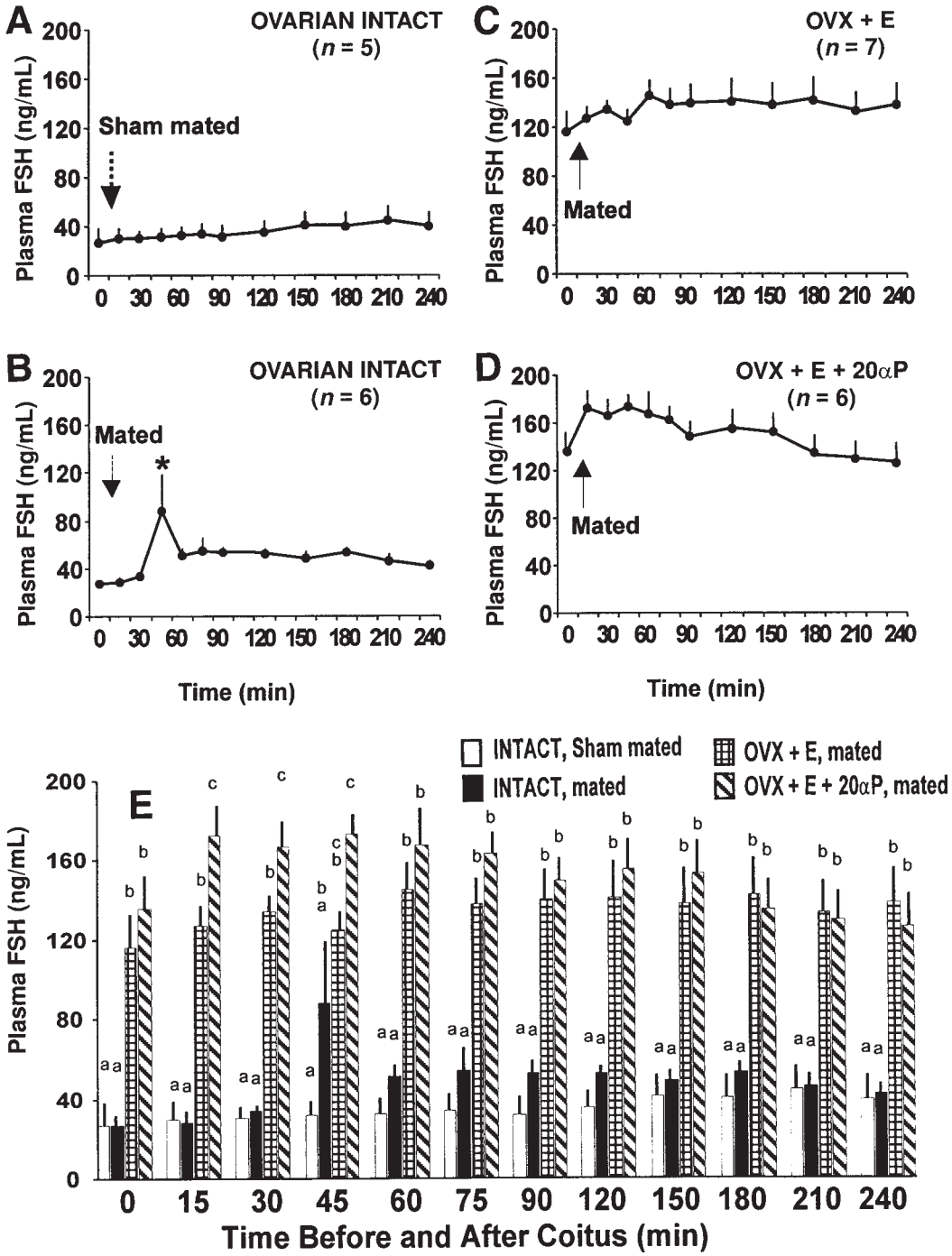

Fig. 2. Mating-induced FSH release in ovarian-intact and OVX rabbits treated with 17β-estradiol (OVX + E) or E and 20α-hydroxyprogesterone (OVX + E + 20αP). *See* Fig. 1 legend for detail.

mediobasal hypothalamus either in2 vitro in the rat *(15)* or in vivo in the rabbit *(16)*. Collectively, these findings are supportive of a facilitatory role of 20αP on LH secretion, but not for the full-scale LH surge after coitus.

We asked the question: could continuous priming (several days) with 20αP in OVX + E rabbits facilitate the coitus-induced LH surge? The data (Fig. 1) clearly suggest that continuous 20αP priming does not restore a full-scale LH surge. It is possible that the hypothalamic-pituitary axis can be better sensitized if 20αP were given in a pulsatile manner. However, limited examination of 20αP pulsatility during estrus in rabbits demonstrated that the amplitude of most 20αP pulses detected during this reproductive stage is very small *(8)*. A more likely conclusion is that ovarian factors other than E and 20αP are involved in the facilitation of the LH response to coital stimulation.

In the present study, plasma FSH levels in OVX + E + 20αP females were briefly elevated above values in OVX

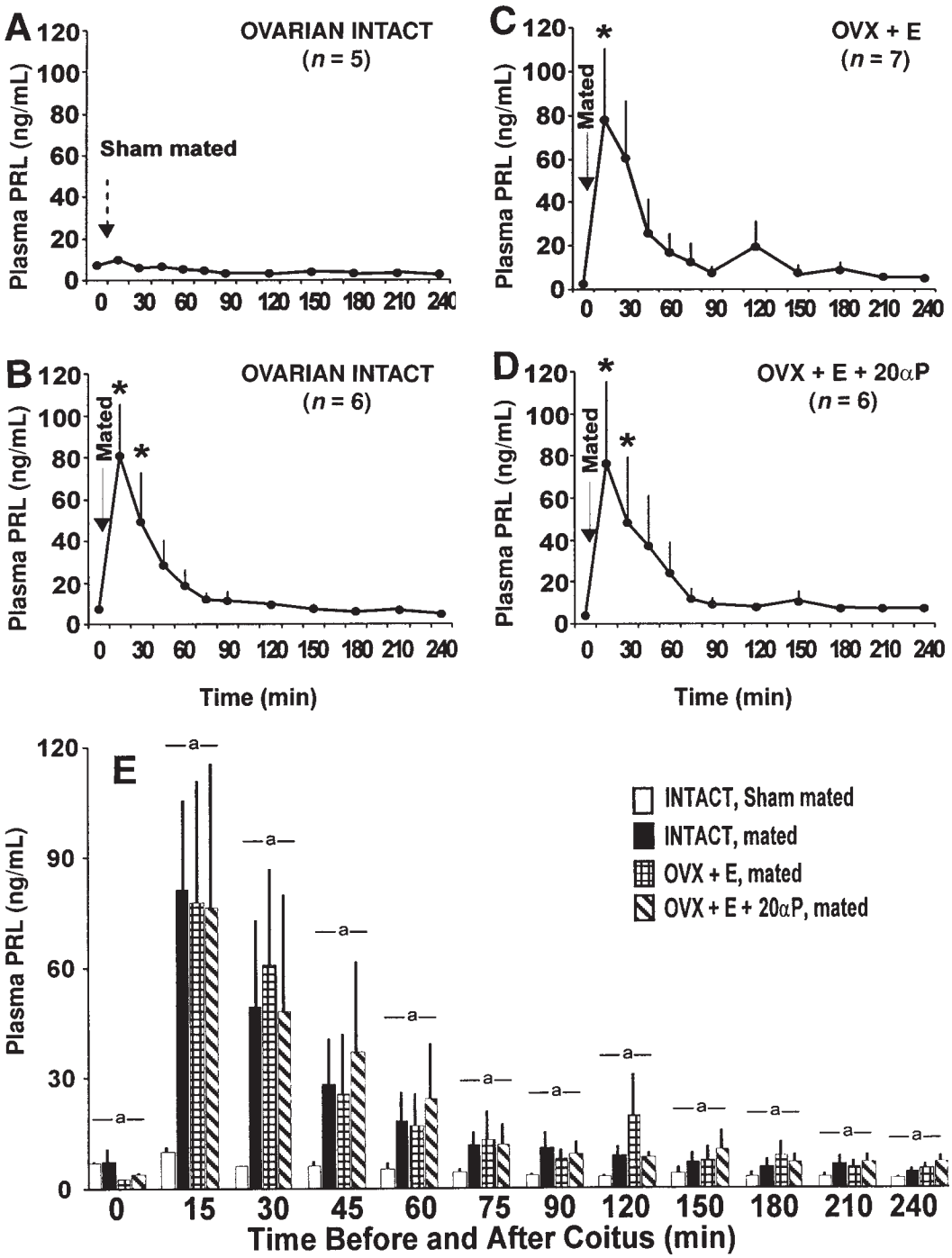

Fig. 3. Mating-induced prolactin (PRL) release in ovarian-intact and OVX rabbits treated with 17β-estradiol (OVX + E) or E and 20α-hydroxyprogesterone (OVX + E + 20αP). See Fig. 1 legend for detail.

+ E animals after coitus. The mechanism by which 20αP elevates FSH in OVX + E rabbits is unknown. In the rat pituitary, progestins are actively interconverted and metabolized by a number of steroidogenic enzymes including 5-α-reductase and 3-α-hydroxysteroid oxidoreductase *(17)*. It has been shown clearly that the allylic steroid 3-α-hydroxy-4-pregnen-20-one, which is found in the rat gonad, pituitary, and hypothalamus, suppresses the GnRH-induced FSH pulses in vitro through a nongenomic mechanism *(17)*. Whether the 20αP treatment in the present study alters the paracrine environment and/or the pituitary FSH response to endogenous pulsatile GnRH secretion remains unresolved. By contrast, FSH levels before and after coitus were elevated in both OVX groups compared with levels in ovarian-intact rabbits. This is consistent with a previous report that basal FSH levels in OVX + E and OVX + E + 20αP animals are two- to three-fold higher than FSH levels in ovarian-intact rabbits *(8)*. Moreover, basal FSH levels in

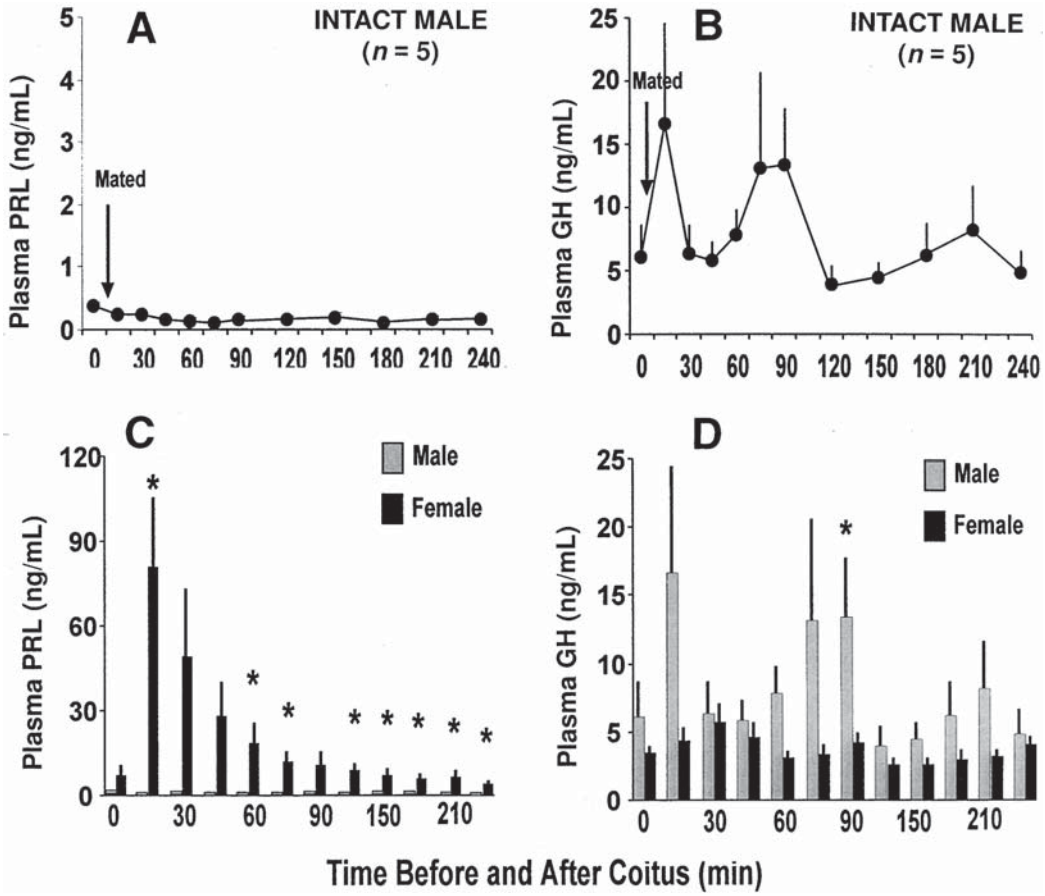

Fig. 4. Profiles of circulating PRL and GH in gonadal-intact male rabbits after coitus **(A,C)**. Mean levels of PRL and GH in gonadal-intact females (data from Figs. 3 and 5) are presented in **(B)** and **(D)** to facilitate comparison with those in gonadal-intact males before and at different time points after coitus. Note the changes in the scale of PRL between (A) and (B). Asterisks indicate a significant difference ($p < 0.05$) in mean hormonal levels between males and females.

OVX rabbits without steroid replacement are 9- to 10-fold higher than FSH levels in intact females *(9)*. Thus, it is clear that basal FSH secretion is maintained at low levels via a combination of steroids and other ovarian factors.

We speculate that the unidentified ovarian factor or factors may exert a dual action on the hypothalamic-pituitary axis, i.e., inhibition of FSH and facilitation of LH. One possible candidate is the ovarian inhibin/activin family of proteins. The inhibition of FSH by inhibins is well established *(see* ref. *18* for a review*)*. Activin has been shown to stimulate GnRH release in vitro, either from rat hypothalamic explants *(9)* or from the immortalized GnRH-secreting tumor cells *(19)*. The data presented herein (Fig. 6) are the first to show that activin also stimulates hypothalamic GnRH release in vivo. Activin-expressing neurons have been found in the rat hypothalamus where GnRH neurons are located *(20)*. Collectively, these findings (*18–21*; Fig. 6) provide the basis for an untested hypothesis that ovarian proteins sensitize the hypothalamohypophyseal axis to produce a full-scale LH surge after coitus. We speculate that the regulation of LH/FSH by activin/inhibin may be particularly important in an induced ovulating species. Female rabbits during the breeding season are maintained at an estrous stage by constant low levels of E and are ready to mate with a male *(10)*. Without the production of the preovulatory E surge, which reflects the optimal condition of preovulatory follicles in spontaneous ovulating species, the rabbit may use other ovarian mechanisms to ensure that maturing eggs in less than optimal conditions do not ovulate after coitus.

Our laboratory has reported previously that male rabbits do not exhibit a GnRH/LH surge after coitus *(4)*. We now report, for the first time, that male rabbits also do not exhibit a PRL surge after coitus (Fig. 4). This is in sharp contrast to our previous *(3)* and current findings (Figs. 3 and 4) that enhanced PRL secretion follows copulation in ovarian-intact females, to findings in the male rat that mating stimulates a PRL release *(22,23)*, and to plasma GH levels that showed no change after coitus in either the male or female (Figs. 4 and 5). Moreover, our study also extends the earlier observation *(3)* to include OVX + E and OVX + E + 20αP mated females. The physiological role of this sexually dimorphic, coitus-induced PRL surge is unknown, but it is unlikely that PRL has any effect on facilitating the coitus-

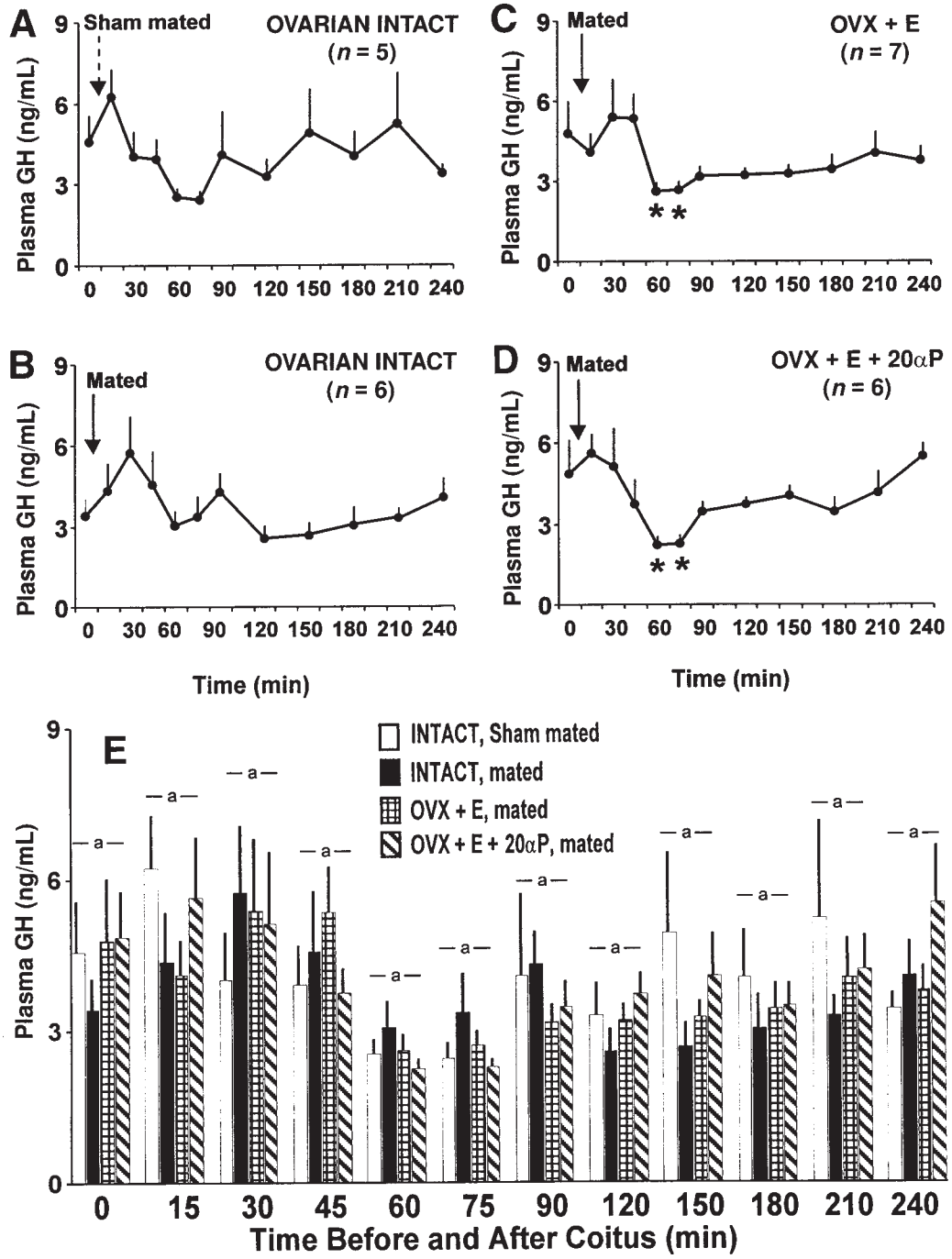

Fig. 5. Mating-induced GH release in ovarian-intact and OVX rabbits treated with 17β-estradiol (OVX + E) or E and 20α-hydroxyprogesterone (OVX + E + 20αP). *See* Fig. 1 legend for detail.

induced LH surge (Fig. 3). It is also associated with neither the reduction of the LH surge, nor the enhancement of FSH levels, in OVX + E and OVX + E + 20αP rabbits (Fig. 3). Prior evidence suggests that PRL may stimulate cholesterol products in the rabbit ovary *(11)*, but whether the coitus-induced PRL surge functions in this capacity is unknown.

The functional and neuroanatomical basis for the striking sexual dimorphism in the coitus-induced LH and PRL surge (*[4]*; the present study) may underscore similar neurochemical mechanisms and pathways in the hypothalamus and brain stem. We previously have shown that the gene expression of tyrosine hydroxylase (the rate-limiting enzyme for norepinephrine synthesis) and norepinephrine transporter (the key protein for norepinephrine reuptake) increases within 30 min after coitus in the locus coeruleus (one of the major noradrenergic areas in the brain stem) in female (but not in male) rabbits *(24)*, suggesting a sexually dimorphic brain stem norepinephrine-hypothalamic GnRH neural system. Furthermore, we recently observed by

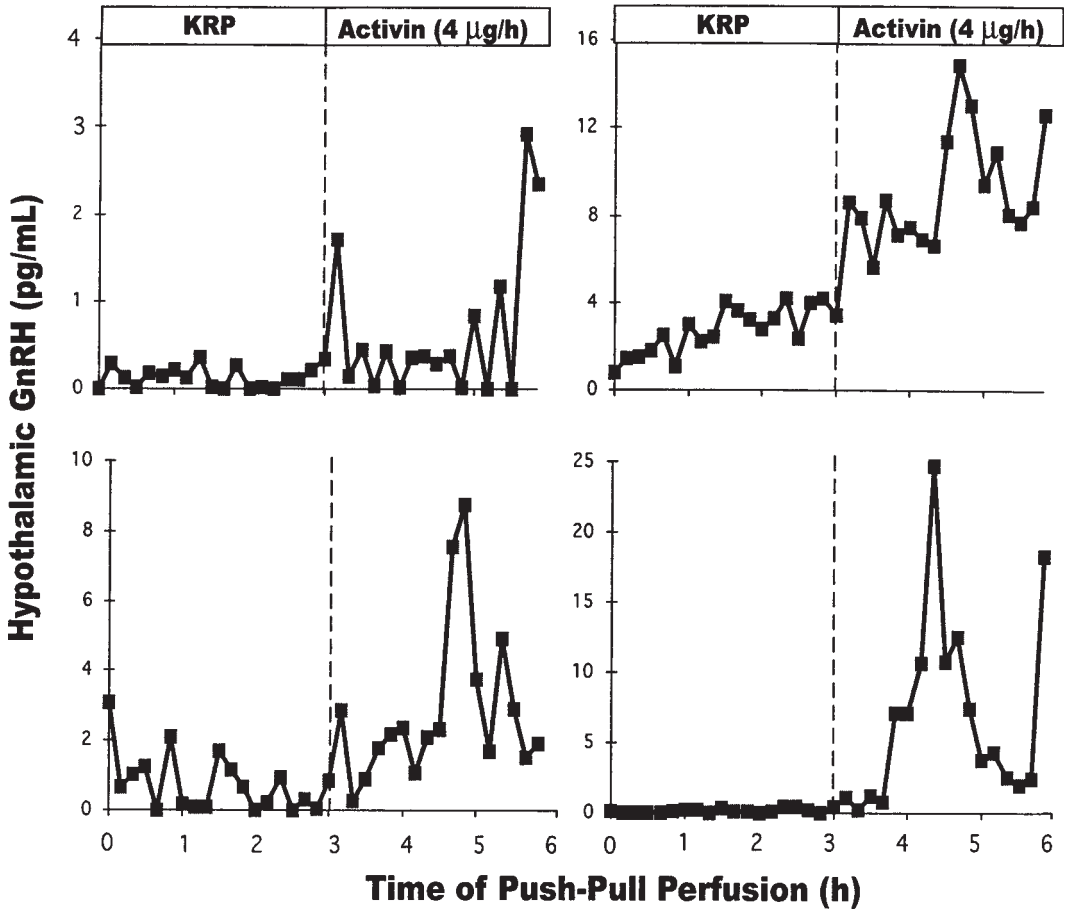

Fig. 6. Stimulation of hypothalamic GnRH release by central administration of activin in OVX rabbits. Krebs ringer phosphate buffer (KRP) was used as both the carrier for activin and artificial cerebrospinal fluid for the push-pull perfusion. Note the changes in the scale for hypothalamic GnRH concentrations among different animals.

double immunocytochemical staining that the coital signaling also involves two other noradrenergic cell groups in the dorsal (A_2) and lateral (A_1) tegmental tract at the caudal medulla oblongata *(25)*. In addition, we have demonstrated that administration of norepinephrine receptor antagonists either blocks basal GnRH secretion *(26)* or attenuates the coitus-induced GnRH surge *(27)* in female rabbits. Collectively, these findings support the hypothesis that the brain stem–hypothalamic norepinephrine system is at least partially involved in the generation of a coitus-induced GnRH/LH surge in the female rabbit. Whether this norepinephrine system also underscores the coitally induced PRL surge in the female rabbit is unknown. However, the fact that neither a GnRH/LH surge nor a PRL surge occurs in the male rabbit after coitus suggests that this neuroanatomical region should be investigated for gender differences.

In the rat, central administration of clonidine, a noradrenergic agonist, stimulates PRL release *(28)*. Other neural components, such as the tuberoinfundibular dopaminergic system, may also be involved in the sexually dimorphic initiation of the PRL surge after coitus because the inhibition of PRL by this hypothalamic dopaminergic system appears to be more profound in male than female rats *(29)*. More recently, a PRL-releasing peptide (PrRP) has been characterized in the rat *(30)*. The localization of the PrRP mRNA is exclusively in the brain stem noradrenergic A_2 and A_1 areas where all PrRP cells also express tyrosine hydroxylase *(31,32)*. Anterograde and retrograde tracing studies in both male and female rats show that A_2 and A_1 PrRP neurons project to the endocrine hypothalamus, particularly the paraventricular nucleus *(32)*. Although the physiological role of the PrRP remains to be determined, we suggest that the coitus-induced PRL surge in the female rabbit (Figs. 3 and 4) may be related to the activation of the brain stem noradrenergic areas *(3,4,24,25,27)*, which enhances PrRP synthesis and release.

In summary, we demonstrated that chronic E and 20αP priming in OVX rabbits cannot restore the full-scale LH surge that is observed in ovarian-intact females. Such steroid treatment also cannot fully restore serum FSH levels to the low values observed in ovarian-intact does. A marked and similar PRL release occurs after mating in both ovarian-intact and OVX steroid-treated females (but not in males), whereas GH secretion does not change during this brief postcoital interval in either sex. Neither changing PRL

nor unchanging GH secretion causes observable alterations in the diminished LH surge after coitus in OVX , steroid-treated animals, but central administration of activin stimulates hypothalamic GnRH release in OVX females. We therefore propose that ovarian protein factors other than E and 20αP may be required for sensitizing the hypothalamic-pituitary axis in response to coital stimulation.

Materials and Methods

Animals

Adult New Zealand White rabbits (3.5–4.5 kg) were obtained from Western Rabbitry (Philomath, OR). The animals were caged individually in a temperature-controlled (22 ± 2°C) and light-regulated (lights on, 7:00 AM to 7:00 PM) room and provided with Purina Rabbit Chow (Ralston, NJ) and tap water ad libitum.

Experiments in Females

Each doe received a femoral vein catheter prior to the first mating trial. Surgical anesthesia was induced with an im injection of ketamine (30 mg/kg of body weight) and xylazine (5 mg/kg of body weight) and maintained by iv injection of the same drug mixture as needed. An indwelling catheter (PV-6; Bolab, Lake Havasu City, AZ) was inserted into the inferior vena cava via the femoral vein to a level below the renal bifurcation. The catheter extension was routed subcutaneously to a position in the lumbar region and connected to a tubing adapter (18 g; Becton-Dickinson, Rutherford, NJ) that had been concealed by medical adhesive silicone (Silastic; Dow Corning, Midland, MI). Following surgery, rabbits were given an im injection of antibiotics (Buprenex; Reckitt & Colman, Richmond, VA) and returned to their cages for recovery. The patency of the catheters was maintained through twice weekly flushes of heparinized saline (10 IU/mL), and then refilling the line with concentrated heparin (1000 IU/mL) (Porcine; Upjohn, Kalamazoo, MI).

The does were allowed 1 wk for recovery before mating. Mating was accomplished by placing the female into the cage of a vasectomized male for 10 min. Coitus usually occurred within 2–5 min. Successful mating, i.e., male ejaculation in the female vagina, was visually judged by the distinct rigid gesture of the male followed by falling off the female's back. Previous studies examining for postcoital sperm in the vagina *(13)* and preovulatory release of GnRH and LH after coitus *(3,4)* confirmed that this visual judgment is 100% accurate in the classification of successful mating in our laboratory. Blood samples were collected before and after coitus as described subsequently.

Twelve female rabbits were used in the first mating trial, in which seven mated. Blood samples were taken successfully in six mated (ovarian-intact group, $n = 6$) and five nonmated (sham-mated group, $n = 5$) individuals. Between 21 and 23 d after completion of the first mating trial, the seven mated rabbits were bilaterally OVX through a midline incision and implanted subcutaneously in the abdominal region with either one 17β-estradiol-filled Silastic capsule (E, $n = 3$) or one E-filled capsule and one 20αP capsule (E + 20αP, $n = 4$). The anesthetic regimen was similar to that already described. Both steroids were purchased from Sigma (St. Louis, MO). Each capsule was made with Silastic medical-grade tubing (1.6 mm id × 3.2 mm od; Dow Corning) and sealed at both ends with silicone glue. All E capsules were 8 mm long, and all 20αP capsules were 40 mm long. Postsurgery rabbits were again given im injections of antibiotics and allowed 15 d for recovery before the second mating trial.

One week following the second mating trial, each rabbit's hormone regimen was switched, so that those previously receiving E alone were given an additional 20αP implant subcutaneously ($n = 3$), and those previously receiving both hormones had the 20αP implant removed ($n = 4$). All anesthetic and postoperative treatments were as already described.

The third mating trial was performed 15 d after the switch of steroid implants. Blood samples were successfully collected in seven rabbits containing an E implant (OVX + E, $n = 7$) and in six rabbits containing both E and 20αP implants (OVX + E + 20αP, $n = 6$).

Blood samples were taken before each of the mating trials (presample) as well as at d 5 following each hormone implantation or switch. In each of the three mating trials, the does were mated with vasectomized bucks and then placed in a special Plexiglas box to facilitate peripheral blood sampling. Box construction (50 cm long × 18 cm wide × 35 cm high) restricted body rotation but allowed access to food and water. Sequential 1.5-mL blood samples were collected at 15-min intervals for 90 min, and then at 30-min intervals for an additional 150 min. Samples were obtained through the femoral vein catheter via a polyvinyl extension set filled with heparinized saline, and then placed in ice-cold heparinized glass tubes. After centrifugation at 4°C for 25 min (1500*g*), plasma was harvested and stored at –14°C until assayed for pituitary hormone and steroid content.

Experiments in Males

To assess whether coitus induces a PRL or GH surge in the male, gonadal-intact male rabbits ($n = 5$) were fitted with a vein catheter that extended through a swivel/tether system *(3,4)* to facilitate sequential blood sampling at frequent (every 10 min) intervals. This frequent sampling procedure was adapted to more accurately detect minor changes, if any, in PRL and GH secretion after coitus in the male. Blood samples were taken during 1 h before and 4 h after mating with a stud female (OVX-E-treated). Ejaculation occurred within 5 min in all five males.

Activin Infusion Trials

To determine whether ovarian proteins are involved in sensitizing the hypothalamohypophyseal axis, OVX

females ($n = 4$) were fitted with a push-pull cannula in the arcuate–median eminence of the mediobasal hypothalamus *(9,26,33)*. Activin (4 μg/h; provided as activin A and B dimers by Genentech, San Francisco, CA) was infused through the push-pull system for 3 h after the initial infusion of carrier (Krebs ringer phosphate buffer) *(33)* for 3 h. Concentrations of GnRH in push-pull perfusate before and during activin infusion were measured by RIA.

Assays

The RIA procedure was the same as previously reported *(9,25,32)*. Plasma LH, FSH, PRL, and GH values were measured by homologous rabbit RIA kits provided by Dr. Al Parlow, Director of the Pituitary Hormone Program, National Institutes of Health (NIH). The final dilution of antiserum for LH, FSH, PRL, and GH assays was 1:2,160,000, 1:72,000, 1:270,000, and 1:630,000, respectively. The intra- and interassay coefficients of variation (CVs) for each of the four hormonal assays were less than 10 and 15%, respectively. For GnRH, the antiserum (EL-14) was diluted to 1:504,000, and the assay sensitivity was 0.2–0.5 pg/tube. The intra- and interassay CVs were estimated as 10 and 15%, respectively.

Plasma values of E and 20αP were quantified by RIA as described earlier *(9)*. The antibody for E, GDN-244, was provided by Dr. Gordon Niswender, Ft. Collins, CO, and was used at a concentration of 1:60,000. The antibody for 20αP, GDN-485, also provided by Dr. Niswender, was used at a 1:250 dilution. The intra- and interassay CVs for both assays were less than 10 and 19%, respectively.

Statistical Analyses

Hormonal changes before and after coitus for each of the four hormones (LH, FSH, PRL, and GH) in each of the four female groups (sham-mated ovarian-intact, $n = 5$; mated ovarian-intact, $n = 6$; OVX + E, $n = 7$; and OVX + E + 20αP, $n = 6$) were analyzed by one-way analysis of variance (ANOVA) with repeated measurements followed by the least significant difference test *(34)*. For each hormone in each treatment, hormonal values after coitus that were significantly ($p < 0.05$) higher or lower than precoital values were indicated by asterisks on the graphic plot. The differences in mean hormonal levels before and at each time point after coitus were analyzed and compared among the four treatment groups by ANOVA and the Tukey's test, respectively. The differences ($p < 0.05$) among the four treatment groups at each time point were indicated by different alphabetic letters (a, b, c) above the bar graphs. In the experiment with males, PRL and GH changes before and after coitus were analyzed by one-way ANOVA with repeated measurements followed by the least significant difference test. Because no change in either PRL or GH was found before or at any time after coitus (*see* Results), the hormonal values were presented at 0, 15, 30, 45, 60, 75, 90, 120, 150, 180, 210, and 240 min for consistency between the two sexes. Comparisons of mean hormonal levels before and at each time point after coitus between gonadal-intact males and females were analyzed by Student's *t*-test. A significant difference ($p < 0.05$) between the male and female was indicated by an asterisk. In the activin study, the mean GnRH values before and during activin infusion in the four rabbits were compared by Student's paired *t*-test. All statistical analyses were performed by the software STATISTIX (Analytical Software, Tallahassee, FL) on a personal computer-based platform.

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