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Received March 31, 1967. P.S.E.B.M., 1967, v126.

Anterior Pituitary Levels of FSH, LH, ACTH and Prolactin After Mating in Female Rabbits.* (32358)

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Copulation induces ovulation in rabbits presumably by stimulating release of hypothalamic neurohumor which causes the anterior pituitary to release ovulating hormone(1). Although the timing of ovulating hormone release from the anterior pituitary gland has been extensively studied in female rabbits (2,3,4), few studies have explored the possibility that copulation may stimulate release of other anterior pituitary tropins. Saxton and Greene(5) provided indirect evidence that copulation altered pituitary level of FSH, LH, ACTH, and TSH in female rabbits. Friedgood and Dawson(6) reported a marked increase in the number of acidophilic carmine cells in rabbit pituitaries 0.5 hour after copulation and rapid degranulation of these cells 3 hours after copulation. These cells are currently believed to be the origin of prolactin(7,8). Based upon this evidence, experiments were designed to test directly the hypothesis that copulation alters pituitary FSH, LH, ACTH and prolactin levels before ovulation.

Materials and methods. Mature female rabbits (8-12 months old) were isolated for at least 35 days in an animal room maintained at 18°C and provided with a 14-hour photoperiod daily. Female rabbits were placed with one of 5 males, allowed to mate, and immediately returned to their home cages. Eight were killed by decapitation at each of the following intervals after mating: 0, 0.25, 0.75, 3, 7, or 11 hours. Anterior pituitaries were removed within 3 minutes after slaughter and immediately trimmed, weighed, and placed in a glass vial directly on Dry Ice.

The 8 pituitaries within each interval after mating were pooled and homogenized in 0.85% sodium chloride in pyrogen free distilled water at 5°C with a glass grinding vessel and Teflon pestle. Homogenates of anterior pituitary tissue were centrifuged at 800 *g* for 10 minutes at 5°C and aliquots of supernatant fluid were stored at —20°C until assayed for hormonal activity. When the first 6 pools of 8 pituitaries had been assayed, the complete experiment was replicated once.

The potencies of FSH, LH, and prolactin were estimated as previously described(9) and modified as follows. FSH potency (ovarian weight augmentation assay(10)) of each pool of pituitaries was measured at 5 and 10 mg-equivalents of fresh anterior pituitary tissue and these were compared with 50 and 100 μg NIH-FSH-S2 and with 30 IU of HCG alone in 21-day rats. LH potency (ovarian ascorbic acid depletion assay(11)) was measured at 0.2 and 0.8 mg-equivalents fresh anterior pituitary tissue and these were compared with 0.4 and 1.6 μg NIH-LH-B2. Five rats were used at each dose level of each unknown and reference preparation for

* Published with approval of Director as Journal Article 4088 from Michigan Agri. Exp. Station. This research was supported by USPHS research grant HD 01374.

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TABLE I. Pituitary FSH After Mating Female Rabbits.

Exp	Hr after mating	Potency*	95% limits
1	0	3.92 ± 1.04	1.86–5.97
	¼	3.91 ± .93	1.48–5.74
	¾	5.26 ± 1.06	3.16–7.36
	3	4.19 ± 1.04	2.12–6.25
	7	2.86 ± 1.03	.82–4.91
	11	4.15 ± 1.04	2.09–6.21
2	0	.74 ± .44	.54–2.32
	¼	1.85 ± .77	.31–3.40
	¾	.96 ± .79	.60–2.54
	3	1.23 ± .78	.33–2.80
	7	1.19 ± .78	.37–2.76
	11	1.95 ± .77	.41–3.49

* Mean ± standard error; μg-equivalents NIH-FSH-S2 per mg anterior pituitary. Each entry derived from an assay of 8 pooled pituitaries. Combined λ was 0.34 and 0.31 for first and second experiments, respectively.

TABLE III. Pituitary Prolactin After Mating Female Rabbits.

Exp	Hr after mating	Potency*	95% limits
1	0	.92 ± .36	.46–1.73
	¼	1.33 ±1.00	.38–4.10
	¾	.22 ± .11	.09– .44
	3	.18 ± .18	.03– .56
	7	.09 ± .02	.01– .53
	11	.95 ± .75	.26–2.90
2	0	.32 ± .23	.08– .83
	¼	.13 ± .12	.02– .38
	¾	.22 ± .17	.05– .59
	3	.22 ± .17	.05– .59
	7	.27 ± .21	.06– .72
	11	.45 ± .32	.13–1.17

* Mean ± standard error; μg-equivalents NIH-P-S5 per mg anterior pituitary. Each entry derived from an assay of 8 pooled pituitaries. Combined λ was 0.28 and 0.31 for first and second experiments, respectively.

FSH and LH assays. Prolactin potency (pigeon crop-sac proliferation assay(12)) was tested at 0.5 and 2 mg-equivalents fresh pituitary tissue and these were compared with 1 and 4 μg NIH-P-S5 (17 IU/mg) with eight birds at each point. ACTH potencies were determined (Saffran and Schally (13)) at 50 and 200 μg-equivalents of fresh pituitary tissue and these were compared with 3 and 12 mU of ACTH (lot no. P1457, Mann Research Laboratories, Inc., N.Y.) with 4 replicate flasks at each point.

Potencies, standard errors of potencies, lambdas, combined slopes, non-parallelism and 95% confidence intervals around potencies were calculated by the methods of Bliss (14).

Results. Average FSH potency (Table I) ranged from 2.02 to 3.11 μg-equivalents NIH-FSH-S2; no significant changes were observed between 0 and 11 hours after copulation. In contrast, average pituitary LH concentration (Table II) decreased continuously from 1.09 μg-equivalents NIH-LH-B2 at copulation to 0.21 μg-equivalents 7 hours later and then increased to 0.63 μg-equivalents by 11 hours.

The greatest decrease in average pituitary prolactin potency (Table III) occurred be-

TABLE II. Pituitary LH After Mating Female Rabbits.

Exp	Hr after mating	Potency*	95% limits
1	0	.74 ± .23	.31–1.49
	¼	.55 ± .16	.24–1.07
	¾	.32 ± .10	.13– .64
	3	.12 ± .04	.04– .27
	7	.10 ± .03	.03– .23
	11	.77 ± .29	.24–1.79
2	0	1.44 ± .36	.79–2.25
	¼	1.35 ± .34	.74–2.42
	¾	.98 ± .25	.53–1.74
	3	.84 ± .21	.45–1.49
	7	.32 ± .08	.16– .59
	11	.49 ± .13	.25– .88

* Mean ± standard error; μg-equivalents NIH-FSH-B2 per mg anterior pituitary. Each entry derived from an assay of 8 pooled pituitaries. Combined λ was 0.19 and 0.22 for first and second experiments, respectively.

TABLE IV. Pituitary ACTH After Mating Female Rabbits.

Exp	Hr after mating	Potency*	95% limits
1	0	.34 ± .19	.09–7.78
	¼	.32 ± .10	.04–8.59
	¾	.13 ± .14	.01– .18
	3	.10 ± .26	.01– .92
	7	.10 ± .01	.07– .14
	11	.39 ± .22	.16–3.19
2	0	.42 ± .20	.08–6.73
	¼	.40 ± .21	.07–5.41
	¾	.09 ± .10	.01– .71
	3	.12 ± .13	.01– .95
	7	.08 ± .01	.04– .12
	11	.43 ± .18	.13–3.25

* Mean ± standard error; units ACTH per mg anterior pituitary. Each entry derived from an assay of 8 pooled pituitaries. Combined λ was 0.13 and 0.16 for first and second experiments, respectively.

tween 0.25 and 0.75 hour (avg 0.73 and 0.22 μg-equivalents NIH-P-B1, respectively) after mating; it remained low until 7 hours (avg 0.18 μg-equivalents) and then increased to copulatory levels by 11 hours (avg 0.70 μg-equivalents) after mating. The replicate experiments were not in good agreement on changes in prolactin potency early after mating, but averages for the two experiments generally paralleled changes in LH. Changes in average ACTH potency (Table IV) resembled those for prolactin. The greatest decrease occurred between 0.25 (avg 0.36 U) and 0.75 hours (avg 0.11 U) after mating; it then remained relatively unchanged to 7 hours (avg 0.09 U), but increased to copulatory levels by 11 hours (avg 0.41 U).

Discussion. These results confirm previous observations that mating causes a rapid decline in pituitary LH concentration in female rabbits. They also demonstrate, for the first time, that mating causes similar declines in prolactin and ACTH. Pituitary concentrations of these 3 tropins remain low until about 7 hours, but largely return to copulatory levels by 11 hours after copulation. Although plasma titers of tropins were not measured in this experiment, decreased pituitary concentrations may reflect concomitant increases in blood plasma(15). Also, Hilliard *et al*(16) demonstrated a rapid increase in 20α-hydroxypregn-4-en-3-one in rabbit ovarian venous effluent which was inversely proportional to levels of pituitary LH reported here.

Hill(2) reported that pituitary gonadotropin stores were maximally depleted within 24 hours after coitus, but the evidence presented here reveals much more rapid disappearance of LH, prolactin and ACTH. In support of the present data, Foster(17) noted maximal degranulation of LH producing basophil cells within 5 hours after coitus.

That the present results failed to substantiate the early observation that mating caused decreased pituitary FSH(5) is presumably due to lack of specificity of early FSH assays. Our data agree with those from Taleisnik *et al*(15) who recently observed that mating caused elevated plasma LH but failed to significantly alter plasma FSH in rats. Granting that exogenous LH alone causes ovulation in rabbits, the present data could be interpreted to suggest that some other pituitary hormones (*e.g.*, prolactin and ACTH) may play a role in ovulation induction or corpus luteum (CL) formation. Furthermore, although prolactin failed to maintain rabbit CL in the absence of the pituitary(18,19), the possibility remains that prolactin or ACTH may facilitate CL function, perhaps synergistically with LH. Of course, rapid disappearance of ACTH from the pituitary could also reflect *normal* stress of mating in rabbits.

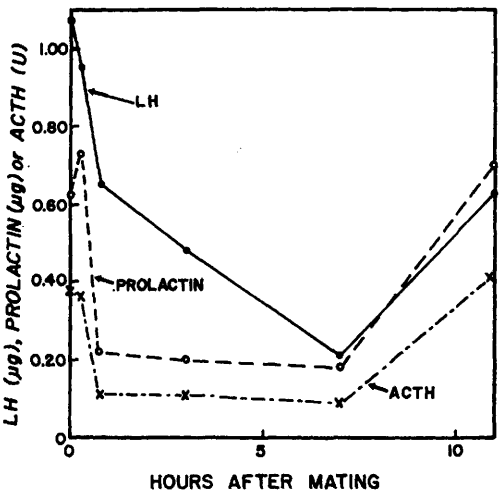

FIG. 1. Concentrations of LH, prolactin, and ACTH per mg pituitary (averages of 2 experiments expressed as μg-or U-equivalents of respective standard hormone).

On the other hand, similarities (Fig. 1) in the dynamics of LH, ACTH and prolactin "release" from the pituitary reveal that the mating stimulus is not specific for LH release. Rather, the results suggest that mating causes release of at least 3 anterior pituitary hormones. Whether post-copulatory release of tropins other than ovulating hormone from the pituitary is purposeful or simply innocently caused by intimate connections between several hypothalamic releasing factor centers remains to be shown.

Summary. Anterior pituitary levels of LH, FSH, ACTH and prolactin were determined at 0, 0.25, 0.75, 3, 7, or 11 hours after mating in 96 female rabbits. Levels of LH, ACTH and prolactin decreased rapidly after mating to low levels which persisted from 0.75 to 7 hours after mating, but copulatory

levels of these tropins were largely restored within 11 hours after mating. Pituitary FSH was not significantly influenced by copulation. The results indicate that the mating stimulus is not specific for ovulating hormone release and suggest that other pituitary tropins may affect reproductive function near the time of ovulation.

The authors express appreciation to Drs. H. A. Tucker and Y. N. Sinha for assistance with prolactin assays, and to Dr. J. Jewel (Ayerst Laboratories) and Dr. R. Zimbelman (Upjohn Co.) for supplies of PMS and HCG, respectively.

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Received May 31, 1967. P.S.E.B.M., 1967, v126.

Antibody Response in Rabbits to Antigenic Stimulation During Amantadine Treatment.* (32359)

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The anti-influenzal effect of amantadine hydrochloride was initially demonstrated by the absence of a serologic response in volunteers challenged with attenuated live Influenza A_2 virus(1). To determine whether the effect could have resulted from drug inhibition of antibody synthesis rather than the prevention of infection, rabbits were treated with amantadine, dextrose, or 6-mercaptopurine and were inoculated with a viral and non-viral antigen.

* This work was done under the sponsorship of the Commission on Acute Respiratory Diseases of the Armed Forces Epidemiological Board, and was supported in part by U.S. Army Medical Research and Development Command, Dept. of the Army, under research contract DA-49-193-MD 2410.

Materials and methods. Animals. Female New Zealand white rabbits weighing 2 kg each were used.

Drug regimen. Amantadine hydrochloride was administered intramuscularly (IM) to 6 rabbits in a dose of 12.5 mg/kg/day in 1.0 ml of normal sterile saline solution and to 6 rabbits intraperitoneally (IP) in a dose of 25.0 mg/kg/day in 1.0 ml of saline solution. As controls, 3 rabbits were given 12.5 mg/kg/day of dextrose in saline solution IM, and 3 received 25 mg/kg/day in 1.0 ml, IP. Six rabbits were given 6-mercaptopurine (6-MP) in a dose of 6 mg/kg/day, intramuscularly. A fresh solution of 6-MP was made each day by dissolving 84 mg of drug in 1.19 ml of 1.0 N sodium hydroxide, which was diluted with 5.81 ml of saline solution.