PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 152, 29-32 (1976)

Regression of Corpora Lutea in the Rabbit after Injection of a Gonadotropin-Releasing Peptide (39320)

R. H. RIPPEL AND E. S. JOHNSON
(Introduced by E. T. KIMURA)

Division of Antibiotics and Natural Products, Abbott Laboratories, North Chicago, Illinois 60064

Normal function of corpora lutea is required throughout pregnancy in the rabbit to insure adequate synthesis and secretion of progesterone. When large doses of exogenous luteinizing hormone (LH) were given to pseudopregnant rabbits (1), ovulation often occurred followed by regression of functional corpora lutea. Subsequently, it was shown that estrogen prevented LH-induced regression of corpora lutea (2, 3) and this steroid is now accepted as the ultimate luteotropic hormone in the rabbit, necessary for maintenance of corpora lutea once they have formed. Recent experiments (4) indicate that the luteotropic stimulus of estrogen reaches the corpus luteum by way of the ovarian blood supply.

The importance of the ovarian follicle as the source of 17β-estradiol was demonstrated when the follicles were destroyed by X-irradiation and the corpora lutea regressed and no longer synthesized or secreted progesterone (5). Moreover, daily injections of estradiol prevented luteolysis caused by the destruction of ovarian follicles. Thus, it was proposed that the luteolytic activity of exogenous LH was secondary to reovulation which removed the source of endogenous estrogen (6).

It was the purpose of this study to determine the influence of [D-Leu^6, des-Gly $NH_2{}^{10}$, Pro-ethylamide^9]-GnRH, and LH-releasing peptide (7, 8), on corpora lutea function in both pregnant and pseudopregnant rabbits.

Materials and methods. Details of the procedures for the synthesis, deblocking, and purification of synthetic [D-Leu^6, des-Gly $NH_2{}^{10}$, Pro-ethylamide^9]-GnRH were described previously (7).

Mature New Zealand does (3.5 to 4.5 kg) were caged individually in a temperature-light controlled room (25°; lights on 6 AM–8 PM) for at least a 3-week period before experimentation. Pseudopregnancy was induced in 12 does by an iv injection of 25 I.U. HCG[1]. All others does were force-mated within a 2-hr period to males of proven fertility and randomly assigned to treatment. They also received an injection of HCG within 5 min of the initial mating. The corpora lutea of pseudopregnancy and pregnancy were marked with India ink on Day 2 to distinguish them from peptide-induced corpora lutea. The peptide was diluted in 0.1% bovine serum albumin and injected into a marginal ear vein. Sesame oil was used as diluent for 17β-estradiol[2] and progesterone, which was administered subcutaneously.

Blood was collected from an ear vein and immediately refrigerated. Within 24 hr the blood samples were centrifuged (2500 rpm) and the serum stored at −20°. Progesterone was measured by a competitive protein-binding assay (9). The rabbits were killed by injecting an air embolism into an ear vein after which the entire reproductive tract was removed and examined for viable and non-viable fetuses. Corpora lutea were removed from the ovaries, cleaned of adhering tissue, and weighed on a torsion balance to the nearest 0.2 mg. The data were subjected to analysis of variance and differences among treatments determined by Duncan's multiple range test (10).

Results. Injection of 50 μg of the peptide on Day 11 of pseudopregnancy reduced ($P < 0.01$) the weight of functioning corpora lutea by Day 17 (16.3 vs 5.1 mg) and induced formation of additional corpora lutea (Table I). Estradiol maintained the weight of corpora lutea of pseudopregnancy

[1] Antuitrin "S," Parke-Davis, Ann Arbor, Michigan.

[2] Progynon suspension, Schering Corp., Bloomfield, New Jersey.

of peptide-treated rabbits, but did not affect the weight of the "new" corpora lutea.

A single 50-μg injection of the peptide on either Day 10, 14, or 19 of pregnancy resulted in 34, 84, and 83% fetal resorptions, respectively, compared to 12% in controls, as observed on Day 28. Four rabbits were examined in each group. The presence of two distinct sets of corpora lutea on the ovaries of peptide-treated rabbits suggested that sufficient LH was released from the pituitary to induce ovulation or cause luteinization of existing follicles. Moreover, there was no affect on percentage of fetal resorption in rabbits which did not ovulate after receiving lower dose levels of either 2 or 10 μg of the peptide.

In another experiment, 50 μg of the peptide injected on Day 15 of pregnancy induced formation of corpora lutea and regression of the functioning corpora lutea (Table II). Serum progesterone was reduced ($P < 0.01$) within 3 days of peptide injection but increased again by Day 23, undoubtedly reflecting progesterone secretion by the "new" corpora lutea (Fig. 1). The decline in serum progesterone in controls by Day 23 is consistent with the decline in ovarian production (11) and circulating levels of progesterone (12) during the last third of pregnancy. Fetal resorption determined at Day 23 was 85 and 4.1%, respectively, for peptide-treated and control animals (Table II). Twice a day injection of 31.2 and 125 ng of estradiol into peptide-treated rabbits did not prevent corpora lutea weight loss or fetal resorption. However, at 500 ng, estradiol prevented corpora lutea regression and fetal loss induced by the peptide, resulting in values similar to controls. In contrast, a dose of 2 μg of estradiol twice daily maintained corpora lutea weight in peptide-treated does, but had a negative effect on fetal survival. Progesterone (25 mg, twice daily) maintained both corpora lutea weight and fetal survival at levels comparable to controls.

Discussion. These experiments demon-

TABLE I. EFFECT OF [D-Leu^6, des-Gly NH_2^{10}, PRO-ETHYLAMIDE^9]-GnRH AND ESTRADIOL ON CORPORA LUTEA WEIGHT IN THE PSEUDOPREGNANT RABBIT.

Group[a]	Corpora lutea of pseudopregnancy		Peptide-induced corpora lutea	
	Number	Weight (mg ± SE)	Number	Weight (mg ± SE)
1. Controls	16.0	16.3 ± 1.1	–	–
2. Peptide (P)[b]	11.2	5.1 ± 0.4	12.8	8.2 ± 0.3
3. P + Estradiol[c]	12.8	18.3 ± 2.4	9.5	11.2 ± 0.8

[a] Four animals per group.
[b] Single injection of 50 μg on Day 11.
[c] Twice daily injection of 1 μg of 17β-estradiol beginning on Day 11. All rabbits were autopsied on Day 17.

TABLE II. EFFECT OF ESTRADIOL (E_2) AND PROGESTERONE (P_4) ON CORPORA LUTEA WEIGHT AND FETAL SURVIVAL IN [D-Leu^6, des-Gly NH_2^{10}, Pro-Ethylamide^9]-GnRH-TREATED PREGNANT RABBITS.

	Number of does implanting per number mated	Mean number implants	Mean number viable fetuses	Resorptions (%)	Mean number corpora lutea		Mean corpora lutea weight (mg)	
					Pregnancy	Induced	Pregnancy	Induced
Controls	11/12	9.00 ± 0.73[c]	8.63 ± 0.64	4.1	11.10 ± 0.47	–	20.40 ± 1.30	–
Peptide (P)[a]	12/13	9.50 ± 0.58	1.42 ± 0.60	85	9.53 ± 0.79	14.2 ± 0.86	7.63 ± 1.20	13.2 ± 1.00
P + E_2[b] (31.2 ng)	5/5	6.00 ± 1.10	0.42 ± 0.24	93	7.80 ± 0.43	12.4 ± 0.66	8.10 ± 0.98	14.3 ± 0.36
P + E_2 (125 ng)	4/5	9.00 ± 1.20	2.00 ± 1.20	78	11.80 ± 0.89	11.0 ± 1.83	7.60 ± 2.40	11.3 ± 2.60
P + E_2 (500 ng)	5/5	7.60 ± 0.51	7.00 ± 0.71	7.9	11.60 ± 1.00	8.8 ± 1.30	16.60 ± 1.40	16.4 ± 0.98
P + E_2 (2000 ng)	5/5	10.00 ± 0.89	0.40 ± 0.24	96	10.40 ± 0.85	11.4 ± 0.82	16.70 ± 0.67	14.2 ± 0.41
P + P_4 (25 mg)	7/8	7.30 ± 1.20	7.00 ± 1.30	4.1	8.70 ± 0.80	10.7 ± 1.00	15.80 ± 1.50	16.5 ± 1.20

[a] Single iv injection of 50 μg on Day 15.
[b] Twice-daily injections of E_2 and P_4 sc in sesame oil.
[c] SE of mean.

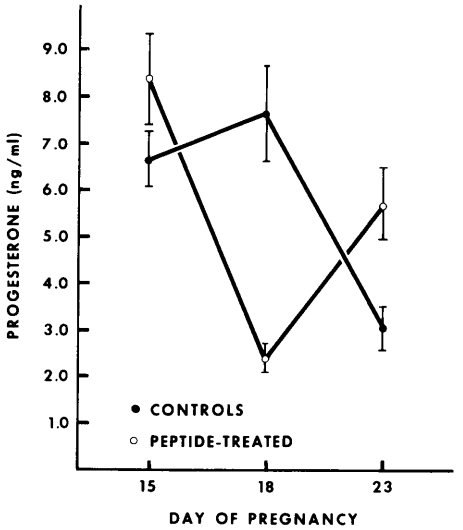

FIG. 1. Serum progesterone concentrations in pregnant rabbits before and after the injection of vehicle or [D-Leu^6, des-Gly $NH_2{}^{10}$, Pro-ethylamide^9]-GnRH. Each point represents the mean ± SE of eight does.

strate that a potent gonadotropin-releasing peptide apparently induces sufficient LH release from the pituitary of the pseudopregnant and pregnant rabbit to cause ovulation and regression of existing corpora lutea. That the peptide induced ovulation in the pregnant rabbit is supported by recent experiments (unpublished) in which we observed ovulation points on the ovary and recovered eggs from the oviducts 24 hr after injection. To our knowledge, this is the first demonstration that endogenously released LH will ovulate the estrogen-secreting follicles present during pseudopregnancy and pregnancy, thus depriving the rabbit of its luteotropic support for existing corpora lutea. In pregnant rabbits, serum progesterone declined within 3 days of peptide injection. Furthermore, corpora lutea regression resulted in a large percentage of fetal resorptions. In many of the rabbits, complete abortion occurred when the peptide was given on Day 19 of pregnancy.

That the luteolytic effect of the peptide is dependent on ovulation is supported by the observations that certain levels of estradiol will maintain corpora lutea weight and fetal viability. Keyes and Nalbandov (5) reported that 2 to 4 μg of estradiol was required daily to maintain function of corpora lutea in rabbits in which the ovaries had been X-irradiated. However, we have found that injecting 0.5 μg of the steroid twice daily maintained both corpora lutea weight and fetal survival. These differences are probably related to the method of destruction of estrogen-secreting ovarian tissue, in that X-irradiation is undoubtedly a more severe treatment than peptide-induced ovulation. In our studies the twice-a-day dose of 2 μg of estradiol maintained corpora lutea weight but was detrimental to fetal survival. The fact that peptide-induced luteolysis was prevented by progesterone is surprising in view of the accepted luteotropic property assigned to estrogen. Possibly the large dose of progesterone protected or sensitized the estradiol receptors of the corpora lutea to the existing low levels of that steroid or allowed for sufficient conversion to estrogen. Verification of these possibilities awaits further experimentation.

Summary. We have shown that a potent agonist of the luteinizing hormone injected on either Day 11 of pseudopregnancy or Day 15 of pregnancy causes luteinization of ovarian follicles and a rapid regression of functional corpora lutea. Serum levels of progesterone declined markedly within 3 days of peptide injection in pregnant animals. In the presence of regressing corpora lutea, fetal resorption occurs which can be prevented by estradiol (500 ng, twice a day) or progesterone. Daily levels of estradiol below 1 μg did not maintain either corpora lutea weight or fetal survival, whereas 4 μg of the steroid maintained corpora lutea weight, but not pregnancy.

The authors wish to thank Ms. Jaxie Dausman and Mr. Joseph Cole for their technical assistance, and Dr. Vernon Stephans for performing the progesterone assays.

1. Stormshak, F., and Casida, L. E., Endocrinology **75,** 321 (1964).
2. Stormshak, F., and Casida, L. E., Endocrinology **77,** 337 (1965).
3. Spies, H. G., Coon, L. L., and Gier, H. T., Endocrinology, **78,** 67 (1966).
4. Holt, J. A., Keyes, P. L., Brown, J. M., and Miller, J. B., Endocrinology **97,** 76 (1975).
5. Keyes, P. L., and Nalbandov, A. V., Endocrinology **80,** 938 (1967).
6. Keyes, P. L., and Nalbandov, A. V., J. Reprod. Fert. **17,** 183 (1968).
7. Fujino, M., Fukuda, T., Shinagawa, S., Kobay-

ashi, S., Yamazaki, I., Nakayama, R., Seely, J. H., White, W. F., and Rippel, R. H., Biochem. Biophys. Res. Commun. **60,** 406 (1974).
8. Rippel, R. H., Johnson, E. S., White, W. F., Fujino, M., Fukuda, T., and Kobayashi, S., Proc. Soc. Exp. Biol. Med. **148,** 1193 (1975).
9. Stevens, V. C., Sparks, S. J., and Powell, J. E., Endocrinology **87,** 658 (1970).
10. Steel, R. G. D., and Torrie, J. H., *in* "Principles and Procedures of Statistics." McGraw-Hill, New York (1960).
11. Hilliard, J., Spies, H. G., and Sawyer, C. H., Endocrinology **82,** 157 (1968).
12. Challis, J. R. G., Davies, I. J., and Ryan, K. J., Endocrinology **93,** 971 (1973).

Received November 24, 1975. P.S.E.B.M. 1976, Vol. 152.