Effect of Estradiol and Progesterone on the LH Release Induced by LH-Releasing Hormone (LH-RH) in Intact Diestrous Rats and Anestrous Ewes[1] (36235)

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Administration of crude or purified luteinizing hormone-releasing hormone (LH-RH) brings about a quick release of LH from the pituitary gland in various species of animals including man (1–4). The magnitude of the LH responses to exogenous LH-RH differs in normal and castrated animals (2), and is influenced also by administration of gonadal steroids (5, 6). Administration of progesterone to intact cycling female rats suppressed but did not abolish the LH release induced by exogenous LH-RH (5). Ovulation induced by intrapituitary infusion of purified LH-RH in estrogen primed rabbits was prevented by pretreatment with progesterone, but this suppression of ovulation could be overcome by raising the dose of LH-RH (7). These data suggest that the suppressive effect of progesterone on the LH-RH induced LH release response depends on the dose of LH-RH used. In contrast to the suppressive effect of progesterone pretreatment, pretreatment of rats and sheep with estrogen seems to augment the LH response to LH-RH (6, 8).

The present study was undertaken to investigate the effect of progesterone, estrogen, and the combined administration of these steroids on LH release induced by large doses of purified LH-RH in intact diestrous rats and anestrous ewes. The possibility of a suppressive activity of progesterone on the LH response to a lower dose of LH-RH has also been studied.

Materials and Methods. In the rat experiments, adult females of the Sprague-Dawley strain (Holtzman) with body weights of 200–290 g were used. They were housed in quarters equipped to provide controlled temperature and illumination (16 hr light and 8 hr darkness) and given free access to Purina Laboratory chow and tap water. Vaginal smears were checked every day in the morning hours. On the morning of the day of estrus, the rats were treated with one of the following preparations: sesame oil, 20 μg of estradiol benzoate (E), 5 mg of progesterone (P), or 20 μg E plus 5 mg P; each preparation was injected subcutaneously in a volume of 0.5 ml. Forty-eight hours after the injection, in the period corresponding to day 2 of diestrus, the animals were divided into 4 groups. They were then anesthetized with 0.65 ml of 25% urethane/100 g body weight. Half of the rats of each group were injected intravenously either with 0.5 ml acidified saline or the same volume of acidified saline containing 1 μg of purified porcine LH-RH (9). One of the groups pretreated with 5 mg P was injected with acidified saline or 50 ng LH-RH. Fifteen minutes after injection, the rats were bled again through the jugular vein. The blood samples were centrifuged and the sera were kept frozen until assayed for LH. LH assays were performed in duplicate by means of the double antibody radioimmunoassay as described by Niswender *et al.* (10). The results were expressed in terms of ng of NIH-LH-S-14/ml. Justification for use of ovine LH to express rat LH was given by Niswender *et al.* (10). The difference between serum LH concentrations before and after the injection of saline or LH-RH was calculated for each animal. The data were subjected to a 2 × 4 or 2 × 2 factorial analysis to determine the interaction between the effects of the different pretreatments and the LH responses to LH-RH or saline.

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TABLE I. Effects of the Pretreatment with Estradiol and/or Progesterone on the Response to 1 μg LH-RH in the Diestrous Female Rat.

Group	Pretreatment	Injection[a]	Mean Serum LH concentration ± SE[b] Before injection	After injection	Serum LH increase, ng/ml ± SE
1	Oil	Saline (12)	0.9 ± 0.1	1.8 ± 0.3	0.5 ± 0.2
		LH-RH (14)	1.3 ± 0.2	16.5 ± 2.5	15.2 ± 2.4
2	Estradiol	Saline (5)	1.4 ± 0.4	2.5 ± 0.4	1.1 ± 0.5
		LH-RH (6)	2.5 ± 1.4	37.4 ± 10.5	34.9 ± 9.5[c]
3	Progesterone	Saline (8)	1.6 ± 0.7	3.1 ± 0.5	1.5 ± 0.5
		LH-RH (8)	2.9 ± 0.6	19.5 ± 2.4	16.6 ± 2.2
4	Estradiol plus progesterone	Saline (7)	0.1 ± 0.1	0.1 ± 0.1	0
		LH-RH (8)	1.0 ± 0.4	6.9 ± 1.2	5.9 ± 1.2[d]

[a] Number of rats in parentheses. [b] Values are ng/ml of NIH-LH-S-14.
[c] LH increase significantly higher than in oil pretreated rats after injection of LH-RH ($p < 0.02$).
[d] LH increase significantly lower than in oil pretreated rats after injection of LH-RH ($p < 0.02$).

Duncan's new multiple range test was also used to compare means among the different groups (11).

In another experiment, two adult white-faced ewes were used. They were maintained under long light to darkness cycles to render them anestrous, and fed a standard diet and water *ad lib*. Control blood samples were obtained from the jugular vein 5 and 10 min after the injection of LH-RH. The ewes were then injected with a combination of 100 μg E plus 50 mg P sc. Forty-eight hours later the response to LH-RH was again investigated in a manner similar to that in the control period. LH was determined in each serum sample in duplicate by means of the double antibody radioimmunoassay method described by Reeves *et al.* (8). NIH-LH-S-14 of ovine origin was used as the standard preparation. Guinea pig anti-ovine LH serum was used as the first antibody. A purified ovine LH obtained from Dr. H. Papkoff was used for iodination with ^{125}I.

Results. Mean serum LH levels before the injection of saline or LH-RH were similar in all rats under study with the exception of those pretreated with EP, which showed lower values (Table I). However, most of the mean values were near the lower limits of sensitivity, which for this assay system are 0.62–1 ng/ml. A slight but significant increase in serum LH levels was observed after the injection of saline in rats pretreated with oil, whereas no significant increase was detected after the same treatment in those pretreated with E, P, or EP. Injection of 1 μg LH-RH significantly increased serum LH levels in all rats, as compared with saline controls. However, the increments of serum LH levels following injection of LH-RH in EP and E pretreated rats were different from those observed in the oil and P pretreated rats. In EP pretreated rats, the increment was significantly smaller ($p < 0.02$) than in control group. In P pretreated rats, the increment was similar to that in control rats. Interaction between LH-RH induced LH release and the effects of the pretreatment with E and EP was significant ($p < 0.02$) indicating that 5 mg progesterone plus 20 μg estradiol injected 48 hr previously suppressed the pituitary response to LH-RH. On the other hand, pretreatment with 20 μg estradiol augmented the response to LH-RH, and pretreatment with 5 mg progesterone did not affect the LH release induced by LH-RH. Fifty nanograms of LH-RH brought about a smaller, but highly significant increase in serum LH concentrations in oil pretreated as well as in P pretreated rats. The rise of LH in the group pretreated with P was, however significantly smaller than in the oil pre-

TABLE II. Effects of the Pretreatment with Progesterone on the Response to 50 ng LH-RH in the Diestrous Female Rat.

Pretreatment	Injection[a]	Mean Serum LH concentration ± SE[b] Before injection	After injection	Serum LH increase, ng/ml ± SE
Oil	Saline (6)	0.89 ± 0.23	1.55 ± 0.14	0.66 ± 0.14
	LH-RH (6)	1.19 ± 0.51	5.71 ± 0.92	4.52 ± 1.08
Progesterone	Saline (6)	1.17 ± 0.40	1.65 ± 0.44	0.48 ± 0.12
	LH-RH (8)	1.43 ± 0.16	3.78 ± 0.55	2.34 ± 0.53[c]

[a] Number of animals in parentheses. [b] Values are expressed as ng/ml NIH-LH-S-14. [c] LH increase significantly lower than in oil pretreated rats after the injection of LH-RH ($p < 0.01$).

treated group, as shown by the presence of significant interaction using 2 × 2 factorial analysis ($p < 0.01$) (Table II).

Suppressive effect of combined administration of E and P on LH-RH induced LH release was observed in anestrous sheep also (Table III). EP pretreatment did not modify the resting levels of serum LH, but suppressed the rise of serum LH following injection of 5 μg LH-RH. This effect was more evident in sheep No. 1 than in sheep No. 2. In the latter, 5 min after injection of LH-RH, LH levels were 50% lower than in the LH-RH test performed before EP pretreatment; at 10 min, however, serum LH was only slightly lower than in the control LH-RH test.

Discussion. From the results of this investigation it is evident that porcine LH-RH can release highly significant amounts of LH in rats and sheep. This LH-RH was a highly purified preparation found to be active in *in vitro* and *in vivo* assays (9). The magnitude of the LH response to LH-RH varied depending on the pretreatment that the animals received. Estradiol significantly augmented LH release in response to LH-RH. This effect of estradiol was reported previously from our laboratory (6). In the present investigation, although the doses of estradiol were similar to those used in the previous report (6), a much higher dose of LH-RH was used. Nevertheless, the results were similar in the two studies. Estrogen pretreatment did not change the basal serum LH levels, but it augmented the response to exogenous LH-RH. The magnitude of augmentation was greater in the present experiment.

Progesterone did not suppress the response to 1 μg LH-RH. Similar findings were observed in our other studies using castrated rats (13). These results indicate that the previously demonstrated suppressive effect of progesterone on LH release after LH-RH (5) depends on a quantitative relationship between the dose of progesterone and the dose of LH-RH administered. It is evident that the 5 mg dose of progesterone used in the present experiment was not able to suppress the effect of 1 μg LH-RH on the rat pituitary gland, but on the other hand, it was effective in suppressing the response to a lower dose of LH-RH. This means that the dose of LH-RH is a very important factor in evaluating the suppressive activity of progesterone. It is interesting to note, however, that pretreatment of the rats with a combination of 5 mg of progesterone (the same dose that was found to be ineffective in altering the response to 1 μg LH-RH) plus 20 μg estradiol (which was shown to have augmentating activity), brought about a clear-cut suppression of LH release after 1 μg LH-RH. This effect was also demonstrated in sheep, in which the combined pretreatment showed a suppressive action on the pituitary response to LH-RH. Since resting serum LH concentrations in the ewe did not appear to have been lowered by the pretreatment with estradiol and progesterone, the speculation that the suppressive effect of both progesterone and estradiol is exerted directly on the pituitary gland is probably correct. On the other hand, in the rats, basal serum LH concentrations were lower in EP pretreated animals, but since most of the basal values in the

TABLE III. Responses to LH-RH Before and After the Injection of Estrogen–Progesterone in Anestrous Sheep.

Sheep no.	Time after injection of LH-RH (min)	Serum LH Levels, ng/ml[a]	
		Before EP	After EP
1	0	0.8	0.6
	5	26.55	17.05
	10	57.0	18.33
2	0	not detectable	0.55
	5	51.0	27.38
	10	68.6	57.4

[a] Expressed in terms of NIH-LH-S-14.

different groups of rats were near the limits of the sensitivity of the asssay, a complete evaluation can not be made.

The suppressive effect of the combination of estrogen plus progesterone may have an important physiological meaning, since after ovulation the ovary secretes large amounts of these steroids (14). It is possible then that the decline of serum LH concentration after ovulation is partly due to the simultaneous presence of both steroids. The existence of relatively high levels of estrogens and progesterone after ovulation may lower serum LH concentrations in part by lowering the pituitary responsiveness to LH-RH and in part through reducing pituitary LH content by a negative feedback effect exerted on the hypothalamus, as was suggested by the report by McCann and Ramirez (15).

Summary. The effect of pretreatment with estradiol, progesterone, or a combination of both steroids on the response of serum LH levels to a large dose of LH-RH was investigated. In groups of intact diestrous rats, it was demonstrated that estradiol augmented LH release induced by LH-RH. Progesterone did not block the LH release induced by 1 μg of LH-RH, but suppressed that induced by 0.05 μg LH-RH. In these rats, as well as in intact anestrous ewes, the combination of estradiol and progesterone clearly suppressed the pituitary LH response to LH-RH.

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1. Gay, L. V., Rebar, R. W., and Midgley, A. R., Proc. Soc. Exp. Biol. Med. **130**, 1344 (1969).

2. Schally, A. V., Carter, W. H., Saito, M., Arimura, A., and Bowers, C. Y., Endocrinology **28**, 1747 (1968).

3. Kastin, A. J., Schally, A. V., Gual, C., Midgley, A. R., Bowers, C. Y., and Diaz-Infante, A., J. Clin. Endocrinol. **29**, 1946 (1969).

4. Schally, A. V., Arimura, A., Kastin, A. J., Reeves, J. J., Bowers, C. Y., and Baba, Y., "21. Colloquium der Gesellschaft für Biologische Chemie," p. 45. Springer/Verlag, Berlin (1970).

5. Arimura, A., and Schally, A. V., Endocrinology 87, 653 (1970).

6. Arimura, A., and Schally, A. V., Proc. Soc. Exp. Biol. Med. 136, 290 (1971).

7. Hilliard, J., Schally, A. V., and Sawyer, C. H., Endocrinology 88, 730 (1971).

8. Reeves, J., Arimura, A. A., and Schally, A. V., Biol. Reprod. 4, 88 (1971).

9. Schally, A. V., Arimura, A., Bowers, C. Y., Kastin, A. J., Sawano, S., and Redding, T. W., Rec. Progr. Horm. Res. **24**, 497 (1968).

10. Niswender, G. D., Midgley, A. R., Monroe, S. E., and Reichert, L. E., Proc. Soc. Exp. Biol. Med. **128**, 807 (1968).

11. Steel, R. G. D., and Torrie, J. H., "Principles and Procedures of Statistics." McGraw Hill, New York (1960).

12. Niswender, G. D., Reichert, L. E., Midgley, A. R., and Nalbandov, A., Endocrinology **84**, 116 (1969).

13. Schally, A. V., Parlow, A. F., Carter, W. H., Saito, M., Bowers, C. Y., and Arimura, A., Endocrinology **86**, 530 (1970).

14. Schwartz, N. B., "21. Colloquium der Gesselschaft fur Biologische Chemie," p. 97. Springer/Verlag, Berlin (1970).

15. McCann, S. M., and Ramirez, V. D., Rec. Progr. Horm. Res. 20, 131 (1964).

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