PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 148, 505–507 (1975)

Binding of Human Chorionic Gonadotropin by Rat Ovarian Slices: Dependence on the Functional State of the Ovary[1] (38571)

C. Y. LEE, KAYOKO TATEISHI,[2] R. J. RYAN AND N. S. JIANG

Departments of Molecular Medicine and Laboratory Medicine, Mayo Clinic, Rochester, Minnesota 55901

In a previous report, we have demonstrated that binding of [^{125}I]hLH by rat ovarian slices increased six- to sevenfold following injection with pregnant mare's serum gonadotropin (PMSG) and human chorionic gonadotropin (hCG) (1). The increase in binding of labeled human luteinizing hormone (hLH) or hCG has been shown to be due to an increased concentration of receptors in the ovary, since the binding affinity is similar for both the primed and nonprimed ovaries (2). Using autoradiographic binding techniques, Midgley has shown that binding of gonadotropins (LH, FSH, prolactin) varies with the functional state of the different ovarian structures (3). In this study, we wish to report the effect of PMSG-ovine LH priming on ovarian binding of [^{125}I]hCG and it's correlation with ovarian progesterone concentration.

Materials and Methods. Immature female Holtzman rats were primed with 50 IU of PMSG at 25 days of age and 125 μg of ovine LH (NIH-S17) 56 hr later. At least three rats were sacrificed at each time period. Binding of [^{125}I]hCG by ovarian slices was performed as described before (4). Binding was expressed as a T/M ratio (cpm/g tissue/cpm/ml medium). Nonspecific binding was assessed by control incubations containing an excess (200 IU/ml) of unlabeled hCG. Labeling of hCG (CR 117, kindly supplied by Dr. Robert Canfield, Columbia University) has been described before (2). Progesterone was measured by radioimmunoassay (5) after extraction with redistilled hexane (6). 20-α-Hydroxyprogesterone was shown not to interfere in the progesterone assay.

Results. The effect of PMSG-ovine LH priming on [^{125}I]hCG binding by ovarian slices is illustrated in Fig. 1A. Ovarian binding in terms of a T/M ratio was approximately three after PMSG and before ovine LH (oLH) injection. Increase in binding activity was observed by 4 days after PMSG or approximately 40 hr after oLH. The binding activity continued to increase sharply and reached a plateau 8 days after PMSG. Nonspecific binding measured in the presence of an excess of unlabeled hCG (200 IU/ml) gave a T/M ratio of 0.75 at 11 days after PMSG, while the T/M ratio without addition of unlabeled hCG was 23.8. The T/M ratio remained at approximately 20 for 9 days. The decline in ovarian binding was evident by the 17th day after PMSG and continued dramatically until it reached a T/M ratio of four by the 19th day. The T/M ratio decreased further to 2.8 by day 22. The large standard deviation noted on day 17 (from 11 rats) and day 18 (7 rats) shown in Fig. 1A was due to variations among individual rats in the time of onset of luteolysis. On rare occasion, ovarian binding activity began to decline as early as 15 days after PMSG. On the other hand, binding activity occasionally was maintained at the T/M ratio of 20 until day 21.

Figure 1A also illustrates the effect of PMSG-oLH priming on ovarian progesterone concentrations. The pattern of ovarian progesterone concentrations correlated well with the ovarian binding activity also shown in Fig. 1A. Ovarian progesterone was increased 4 days after PMSG and reached a plateau at day 7. The high level of progesterone was maintained until day 17. A decline was noted by the 18th day and continued until it reached a low level by the 22nd day after PMSG.

The effect of PMSG-oLH injection on ovarian weight is illustrated in Fig. 1B. Ovarian weight increased sharply following PMSG injection and reached a peak of 130–

[1] Supported by Grants and Contracts from USPHS (HD 08099, HD 03726, HD 12232) and the Mayo Foundation.

[2] Rotary International Visiting Fellow.

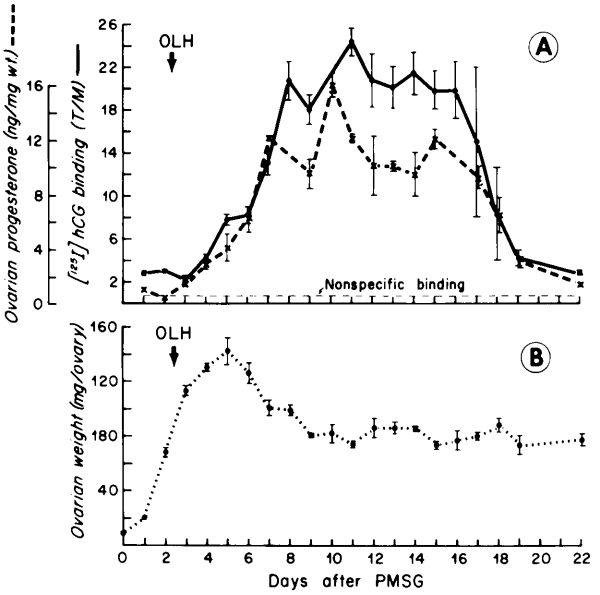

FIG. 1. Daily changes in hCG binding (A), progesterone concentration (A) and weight of rat ovaries (B) following PMSG-ovine LH priming. Binding of labeled hCG (●——●) by ovarian slices was expressed as a tissue/medium ratio. Each bar represents the mean ± SD for three to 11 rats. For both ovarian progesterone concentration (×—×) and ovarian weight (● ||| ●), each bar represents the mean ± SE from three to four rats.

140 mg/ovary by the fourth to fifth day and declined to 80.5 mg/ovary by the ninth day. Ovarian weight remained essentially the same during the subsequent days of observation.

Discussion. The present data indicate that hCG binding activity of rat ovaries undergoes variation that appears to depend upon the functional state of the ovary. In a previous report, we have demonstrated that hLH receptors can be induced by PMSG-hCG injection (1). Priming of immature rats with PMSG-hCG had no influence on the dissociation constant of [^{125}I]hLH binding to ovarian receptors, while the receptor concentration increased 64-fold per ovary after priming (2). In this report we have demonstrated that ovarian slices are capable of accumulating labeled hCG to a T/M ratio of approximately 20 after PMSG-oLH injection. The high binding activity of heavily luteinized ovaries was followed by a rapid decrease to prepriming levels during luteolysis.

The similar patterns of ovarian binding activity and ovarian progesterone shown in Fig. 1A suggest that binding activity is closely associated with the formation and the luteolysis of corpora lutea. Goldberg *et al.* (7) have shown that maximal stimulation of [^{3}H]hCG uptake *in vivo* was accompanied by extensive corpus luteum formation. The simultaneous decline in ovarian binding activity and ovarian progesterone during luteolysis illustrated in Fig. 1A is of interest. The decrease in ovarian hCG binding activity during luteolysis appears due to a decreased concentration of receptors and not due to decreased binding affinity (unpublished observation). Estrogen treatment which prevented luteolysis, as evidenced by sustained high levels of progesterone in both the blood and ovary, also prevented the decline in ovarian binding of [^{125}I]hCG (4). These data suggested that the loss of hCG receptors is a biochemical event related to luteolysis. On the other hand, luteolysis is not accompanied by the loss of ovarian weight as shown in Fig. 1B, nor changes in DNA content and DNA synthesis (8).

In this study, utilizing ovarian slices, no attempt could be made to differentiate function of the corpus luteum from function of the ovarian interstitium. Thus, it is possible

that the duration of the effects noted above could represent an early contribution (up to days 5–8) from the corpus luteum that then regressed but were masked by later effects which occurred in the interstitium and persisted until day 17 or so. HCG has been shown to bind to ovarian interstitial tissue in the pseudopregnant rat, using autoradiographic techniques, but the binding activity was relatively low compared to luteal tissue (3).

Summary. Ovarian receptors specific for hCG-LH can be induced by PMSG-ovine LH priming. The marked increase in binding activity was accompanied by a rise in ovarian progesterone concentration and preceded by ovarian weight increase. The high binding activity of heavily luteinized ovaries was followed by a decline to prepriming levels during luteolysis. The ovarian progesterone content was decreased simultaneously and no change in ovarian weight was evident. The data demonstrate that ovarian hCG binding activity undergoes variation which depends on the functional state of ovaries.

1. Lee, C. Y., and Ryan, R. J., Endocrinology **89,** 1515 (1971).
2. Lee, C. Y., and Ryan, R. J., Biochemistry **23,** 4609 (1973).
3. Midgley, A. R., Jr., *in* "Receptors for Reproductive Hormones" (B. W. O'Malley and A. R. Means, eds.), p. 365, Plenum Press, N. Y. (1973).
4. Lee, C. Y., and Ryan, R. J., Endocrinology, **95,** 1691 (1974).
5. Lee, C. Y., Coulam, C. B., Jiang, N. S., and Ryan, R. J., J. Clin. Endocrinol. Metabol. **36,** 148 (1973).
6. Swain, M. C., Clin. Chim. Acta **39,** 455 (1972).
7. Goldenberg, R. L., Reiter, E. O., Vaitukaitis, J. L., and Ross, G. T., Endocrinology **92,** 1565 (1973).
8. Bartosik, D., Szarowski, D. H., and Stone, M. L., Proc. Soc. Exp. Biol. Med. **145,** 845 (1974).

Received September 6, 1974. P.S.E.B.M. 1975, Vol. 148.