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The Binding of ^{131}I-HCG to Mature Follicles Isolated from the Rabbit Ovary[1] (37828)

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Recent reports have shown that steroid synthesis by rabbit ovaries (1) and rabbit ovarian follicles (2) declines to virtually unmeasurable levels just prior to ovulation. This cessation in steroid production is reflected by a preovulatory fall in the blood levels of estrogen (3) as well as other steroids (1, 3). Blood gonadotropin levels are also low in the peri-ovulatory period (4, 5), but exogenous replacement of these gonadotropins does not return the lost steroidogenic capacity to the tissues (1, 2). We have undertaken a study of the binding of gonadotropin to follicular tissue in order to determine whether any decrease in this binding could account for the steroidogenic shutdown at the time of ovulation.

Materials and Methods. The general methods used in these studies for mating, dissecting follicles, and incubation of follicles have been previously described (2, 6, 7). Human chorionic gonadotropin (HCG) was purchased from Schwarz Mann (sp act 4600 IU/mg), and luteinizing hormone (LH S16) and follicle-stimulating hormone (FSH S8) were obtained from the National Institute of Arthritis and Metabolic Diseases. The iodination of HCG was performed by the method of Greenwood *et al.* (8) modified to yield a specific activity in the range of 30–100 μCi/μg.

The specific binding of ^{131}I-HCG to follicles was measured by simultaneous incubation of 2 sets of follicles from each rabbit. Since control and experimental follicles were obtained from the same ovaries, each animal served as its own control and differences between rabbits were thereby minimized. In these studies, 3–6 follicles were incubated for 30 min with the gonadotropin, while the same number of follicles were incubated with an equal concentration of bovine serum albumin (BSA) as control. After this 30-min preincubation, approximately 10^6 dpm of ^{131}I-HCG was added to each incubation vessel, and they were returned to the incubator for an additional 10 min. At the end of the incubation, the medium was discarded and the follicles washed 6 times in 1 ml of buffer; after each wash, the amount of ^{131}I retained by the follicles was counted (Packard Gamma Spectrometer). In another series of studies, both sets of follicles were coincubated for 30 min with ^{131}I-HCG in the presence of unlabeled HCG or BSA. These incubations were followed by the same series of 6 washes and counts as previously described. There was no apparent difference in the results obtained by these 2 experimental techniques. All washings were performed in plastic tubes, and these were discarded after each wash.

The results are expressed as "% of bound ^{131}I HCG." In this expression, 100% represents the level of binding to control follicles, and any inhibition due to the addition of unlabeled gonadotropin yields values of less than 100%. Statistical analysis was performed using a paired *t* test.

Results. Six successive washings of incu-

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bated follicles with buffer cause a rapid removal of most of the unbound ^{131}I-HCG. Figure 1 shows average values for 6 sets of follicles, half of them incubated with 5 μg HCG/ml, and the remaining follicles from the same animals incubated with 5 μg BSA/ml. It is evident from Fig. 1 that by the 4th wash, little further radioactivity washes off and the percent of the total ^{131}I-HCG bound remains constant thereafter.

In another experiment not depicted here, follicles were cut in half with microscissors and then incubated with ^{131}I-HCG. The cut follicles bound 0.14 ± 0.03% of the available ^{131}I HCG/follicle, while uncut control follicles bound 0.10 ± 0.005%/follicle. This 42% difference in the means of the 2 groups proves to be statistically significant ($P = 0.05$).

Coincubation (Expts A and B) or preincubation (Expt C) of matched sets of follicles with ^{131}I-HCG plus varying amounts of HCG or BSA results in a partial inhibition of ^{131}I-HCG binding (Fig. 2). In all three of the experiments depicted, little or no inhibition of ^{131}I-HCG binding at 0.005 and 0.05 μg HCG/ml can be seen; maximal inhibition occurs at 5.0 and 50 μg HCG/ml. It has not been possible to further reduce the ^{131}I-HCG binding from the level seen after incubation with 5.0 μg HCG/ml; the remaining 50–55% is apparently nonspecific or irreversible binding. In the range of 5.0 to 0.05 μg HCG/ml, the relationship between gonadotropin concentration and binding appears to be linear.

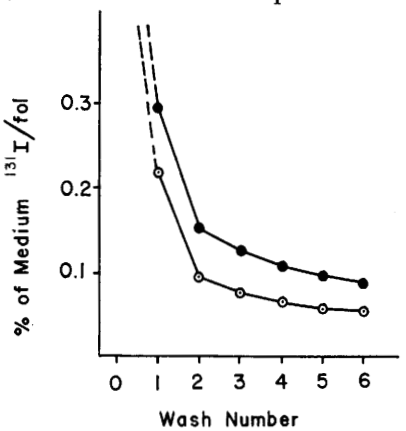

FIG. 1. The effect of sequential washings with buffer on the binding of ^{131}I-HCG to isolated ovarian follicles. Closed circles (●) indicate percent of medium level of radioactivity/follicle after incubation with BSA (5 μg/ml). Open circles (○) indicate percent of medium level of radioactivity/follicle after incubation with HCG (5 μg/ml). Each point represents the mean of 6 paired determinations.

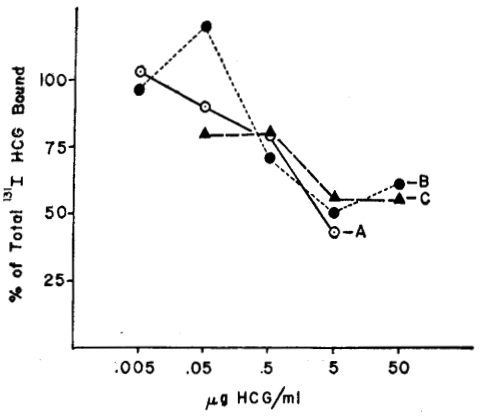

FIG. 2. The effect of incubation in the presence of unlabeled HCG on the binding of ^{131}I-HCG by isolated ovarian follicles. Each point in Expts A and B is the result of coincubation of follicles with ^{131}I-HCG plus unlabeled HCG or BSA. The points in Expt C are the averages of 4–11 separate preincubations with unlabeled HCG or BSA followed by incubation with ^{131}I-HCG.

The results in Table I show that ^{131}I-HCG binding can be inhibited by preincubation of follicles with 5 μg/ml of LH. However, the same amount of FSH does not cause any decrease in the level of ^{131}I-HCG binding. This finding suggests that in the isolated follicle, HCG may occupy the same binding site as LH; FSH, on the other hand, appears to be bound at some other site.

While the biological activity of LH has been demonstrated in isolated rabbit follicles (2, 6), HCG activity has not been determined in this system. To test the activity of HCG, follicles were incubated with ^{14}C-methionine plus 0.5 μg HCG/ml and compared to methionine incorporation by control follicles. After incubation, follicular proteins were precipitated and mass determined by the method of Lowry *et al.* (9); an aliquot was counted for radioactivity. The results show that 0.5 μg HCG/ml

TABLE I. The Effect of Preincubation with LH or FSH on the Binding of ^{131}I-HCG to Follicles.

Gonadotropin	Gonadotropin concentration (μg/ml)	Percent of total ^{131}I-HCG bound
LH S16	5	72.0 ± 5.4 (6)[a,*]
FSH S8	5	104.3 ± 7.8 (7)
HCG	5	57.6 ± 3.0 (11)*

[a] Mean ± SEM (No. of observations in parentheses).

* $P < 0.05$ when gonadotropin and control incubations compared by paired variable t analysis.

caused a 65.6 ± 32% increase in methionine ^{14}C incorporation into follicular protein. This increase proves to be statistically significant ($P < 0.05$).

Table II presents the results of ^{131}I-HCG incubations of follicles from mated rabbits. By selecting follicles from unmated does as well as does 2, 6, and 12 hr after mating, a full range of pre- and postovulatory follicles were examined. The results show that there is significant binding of ^{131}I-HCG to follicles from all time intervals. However, no change occurs in the total binding of ^{131}I-HCG in any of these groups. The average percent of total binding values for preovulatory follicles (2 and 6 hr) and postovulatory follicles (12 hr) do not differ from the precoital level (0 hr) or from one another.

Discussion. Many studies appear in the literature dealing with the binding of gonadotropic hormones to ovarian tissues. Such studies employ homogenates, subcellular fractions, slices, and pure cell cultures of ovaries. The present investigation deals with the binding of gonadotropin to an ovarian element (i.e, a Graafian follicle) which has been left structurally intact and yet freed of contamination by interstitial or luteal tissue. Use of whole follicles maintains the *in vivo* state with granulosal cell layers separated from the gonadotropin containing the incubation medium by a basement membrane and thecal cells in direct contact with the HCG-containing buffer. A failure to keep the structural integrity of the follicle could yield misleading binding results. In rat ovaries, HCG has been found to bind to granulosal cell layers in only some medium and large follicles; thecal cell layers, on the other hand, always bind HCG (10). In the present experiment, cut follicles were incubated with ^{131}I-HCG, thereby exposing granulosa cells directly to the gonadotropin. These cut follicles bound an average of 40% more HCG than uncut follicles, but the binding showed greater variability than that observed in intact follicles. Apparently, rabbit granulosa cells show the same general HCG binding characteristics as whole follicles, but exposure of these cells directly to the gonadotropin may represent an artificial situation that never occurs in the nonovulating follicle. The possibility that ^{131}I-HCG is trapped inside the follicles and that trapping might account for the observed binding is also ruled out by the cut follicle experiment.

TABLE II. The Effect of Mating on the Binding of ^{131}I-HCG to Follicles.

Hours postcoitus	% of total ^{131}I-HCG bound
0	57.6 ± 3.0 (11)[a,*]
2	55.7 ± 7.0 (4)*
6	59.0 ± 4.4 (5)*
12	57.9 ± 5.5 (4)*

[a] Mean ± SEM (No. of observations in parentheses).

* $P < 0.05$ when gonadotropin and control incubations compared by paired variable t analysis.

The amount of ^{131}I-HCG bound/follicle is inversely proportional to the absolute amount of HCG present during the incubation over the range of 0.05 to 5.0 μg HCG/ml (Fig. 2). It appears, however, that 5 μg HCG/ml is a saturating level of the hormone since the percent of binding is not further decreased when unlabeled HCG concentration is raised to 50 μg/ml. The binding at 5 and 50 μg HCG/ml must be nonspecific and may represent the uptake of damaged hormone as has been suggested (11). In addition, some portion of this nonspecific binding could relate to the impurities present in the HCG preparation used.

The threshold of inhibition of HCG binding is in the range of 0.005 to 0.05 μg/ml; the percent of total binding values

were close to 100% in this range, indicating no binding. These values for the threshold are somewhat higher than published values for other tissues. Danzo (12) reported that the threshold of inhibition in luteinized rat ovaries was about 0.002 μg/ml; HCG binding to rat testis homogenates can be calculated to be about this same level (13). The follicular-binding threshold level for HCG are also somewhat higher than the threshold level for gonadotropic stimulation of the follicle; 0.0025 μg LH/ml gave maximal stimulation of steroidogenesis by isolated follicles (6); while in the present experiment, 10–20 times more gonadotropin was required to demonstrate binding. Utilization of an HCG preparation of greater purity might resolve this apparent discrepancy between the HCG binding threshold and the published thresholds for biological activity.

Previously published rabbit studies have shown that addition of exogenous LH to isolated follicles (2) or HCG to ovarian slices (1) will not reverse the cessation in steroid synthesis which occurs at ovulation. It had, therefore, been postulated that the shutdown in preovulatory steroidogenesis was the result of a loss of gonadotropin-binding capacity by the ovulating follicle. In the experiments presented in Table II, follicles were removed from rabbits mated just before (0 hr) and 2, 6, and 12 hr prior to sacrifice; these follicles were tested for HCG binding. It is evident from the results of this study (Table II) that there is no change in the binding of ^{131}I-HCG in any of the preovulatory or postovulatory groups. We therefore conclude that the halt in follicular steroidogenesis which occurs between mating and ovulation cannot be attributed to a failure of the follicle to bind the stimulating agent. Rather, some metabolic blockage in the steroidogenic process must be sought.

Summary. Measurements have been made of the binding of HCG to intact follicles isolated from rabbit ovaries. Competition studies suggest that HCG and LH are bound to the same or overlapping binding sites, while FSH does not appear to compete with HCG for binding. Follicles isolated from unmated, preovulatory, or postovulatory rabbits bind HCG equally well. It is therefore concluded that the cessation in steroidogenesis which occurs just prior to ovulation in the rabbit cannot be attributed to a failure of the follicle to bind the stimulating gonadotropins.

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