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EVIDENCE THAT THE SUBUNIT STRUCTURE OF GONADOTROPIN RECEPTOR IS PRESERVED DURING REGRESSION OF RAT CORPUS LUTEUM

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The level of hCG/LH receptor has been shown to undergo marked changes during the life span of rat corpus luteum. To evaluate whether these fluctuations are due to changes in the receptor subunit structure or receptor protein content, the ^{125}I-hCG binding activity and the receptor subunit structure were determined during different time periods of pseudopregnancy. The maximum ^{125}I-hCG binding activity was observed on day 7, after which it decreased by 20 and 45% on day 11 and day 14, respectively. The Scatchard analysis of ^{125}I-hCG binding data showed that the decrease in binding activity was caused by a change in the number of binding sites rather than a change in the binding affinity. The LH/hCG receptor in ovarian membranes obtained on days 7, 11 and 14 were then characterized by the method of affinity cross-linking. All four subunits of the LH/hCG receptor were detected in the ovarian membranes at all stages while the intensity decreased parallel to a decrease in hCG binding from day 7 to day 14. These results suggest that the decrease in ^{125}I-hCG binding activity in rat ovarian membranes from day 7 to day 14 of pseudopregnancy is due to a decrease in receptor concentration rather than a change in the receptor subunit structure.

It has been shown that the responsiveness to hCG by rat luteal cells obtained at different time periods of pseudopregnancy vary considerably leading to loss of corpus luteum function during luteal regression (1). The responsiveness is parallel to the hCG binding activity of luteal cells during the life span of corpus luteum (2). In this communication, we have examined the kinetics of ^{125}I-hCG binding to the ovarian membranes and the structure of the LH/hCG receptor during different responsive states of the corpus luteum. Our results suggest that the decrease in ^{125}I-hCG binding activity in pseudopregnant rat ovarian membranes from day 7 to day 14 is due to a decrease in receptor concentration rather than a change in receptor structure.

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MATERIALS AND METHODS

Human chorionic gonadotropin (CR-121, 13,450 IU/mg) was generously supplied by Dr. Robert E. Canfield of Columbia University. Collagenase (CLS, Type 1) was obtained from Worthington Biochemical Corp. Pregnant mare's serum gonadotropin, human chorionic gonadotropin, dithiothreitol, ε-amino-n-caproic acid, phenylmethylsulfonyl flouride and N-tosyl-L-lysine chloromethyl ketone were purchased from Sigma. Minimum Essential Medium with Earle's salts (MEM) was obtained from Grand Island Biological Co. Disuccinimidyl suberate was purchased from Pierce Chemical Co. ^{125}I-hCG was prepared by the method of Catt et al. (3). Reagents for electrophoresis were purchased from Bio-Rad. Kodak XR-5 film, lightning-plus intensifying screen and X-omatic cassette (8 x 10 in) were obtained from Eastman Kodak Co. All other reagents used were of analytical reagent grade.

Isolation of Partially Purified Plasma Membranes and Binding Assays. - Sprague-Dawley rats were treated with PMSG and hCG as described by Parlow to obtain pseudopregnant rat ovaries (4). The day of hCG injection is assigned as day 0. Plasma membranes were prepared by the procedure described by Gospodarowicz on day 7, 11 or 14 after hCG injection (5). The binding of ^{125}I-hCG to the membranes was carried out by the method described earlier from this laboratory (6). The slope of the line obtained from Scatchard plot is equal to - 1/Kd (7).

The procedures for preparation of collagenase-dispersed luteal cells, ^{125}I-hCG binding, cross-linking conditions, electrophoresis and autoradiography have been described by us previously (9). Briefly, aliquots of cells (2 x 10^6 cells) were incubated with 0.5 nM ^{125}I-hCG for 2 h at 37°C in the presence or absence of 1.5 μM unlabeled hCG. After removal of the unbound ^{125}I-hCG from the cells by centrifugation and decantation, the cells were washed once and the ^{125}I-hCG-equilibrated cells were suspended in 180 μl of MEM and reacted with 1 mM DSS at 4°C for 15 min. The resulting ^{125}I-hCG-linked luteal cells were solubilized with 2% SDS solution and subjected to SDS-PAGE followed by autoradiography. Protein was determined by the colorimetric procedure of Lowry et al (8).

RESULTS AND DISCUSSION

An examination of the ^{125}I-hCG binding activity during the life span of corpus luteum revealed that the maximum ^{125}I-hCG binding activity was reached on day 7, after which it decreased by 20 and 45% on day 11 and day 14, respectively (Fig. 1). This observation raised the question whether the decrease in ^{125}I-hCG binding activity was due to a change in receptor structure, binding affinity, or a decrease in receptor number. To examine this, the kinetics of ^{125}I-hCG binding to ovarian membranes obtained from pseudopregnant rats on day 7, 11 and 14 were determined. The binding of ^{125}I-hCG to the membranes as a function of the ligand concentration is shown in Fig. 2 inset. As expected, at all the indicated ligand concentrations, the specific binding of ^{125}I-hCG to the ovarian membranes was the highest on

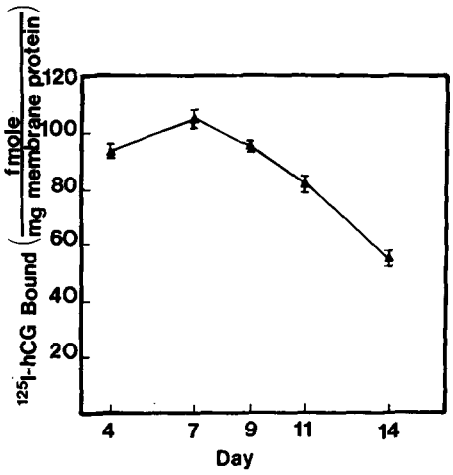

Fig. 1. ^{125}I-hCG binding activity during the life span of corpus luteum. Rats were sacrificed on the indicated day after hCG injection and plasma membrane fraction was prepared from the isolated ovaries. Aliquots of membranes (125 µg membrane protein) were incubated with 0.5 nM ^{125}I-hCG in the absence (total binding) or in the presence of 1.5 µM unlabeled hCG (nonspecific binding) in a final volume of 0.3 ml binding assay buffer. Incubation was carried out at 37°C for 60 min. The specific binding was calculated by subtracting the nonspecific binding from the total binding.

day 7 and the lowest on day 14. There was no difference in the nonspecific binding on days 7, 11 and 14.

Scatchard analysis of the binding data (Fig. 2) was used to determine the Kd and the binding capacity. Three parallel lines were obtained as shown in Fig. 2A, B and C with Kd of 1.25×10^{-10}M for day 7, 1.4×10^{-10} M for day 11 and 1.37×10^{-10} M for day 14. The binding capacity was 130, 104 and 74 fmole per mg membrane protein on day 7, 11 and 14, respectively. The data derived from Fig. 2 are summarized in Table I. These results suggest that the decrease of ^{125}I-hCG binding activity of the ovarian membranes after day 7 of pseudopregnancy was caused by a decrease in the number of binding sites rather than a change in the binding affinity. Experiments were then carried out to characterize the nature of the LH/hCG receptor in luteal cells obtained from pseudopregnant rats of day 7, 11 and 14 by the method of affinity cross-linking as described previously (9) (Fig. 3).

We have previously shown that the LH/hCG receptor is an oligomeric complex composed of four nonidentical subunits linked by disulfide bonds

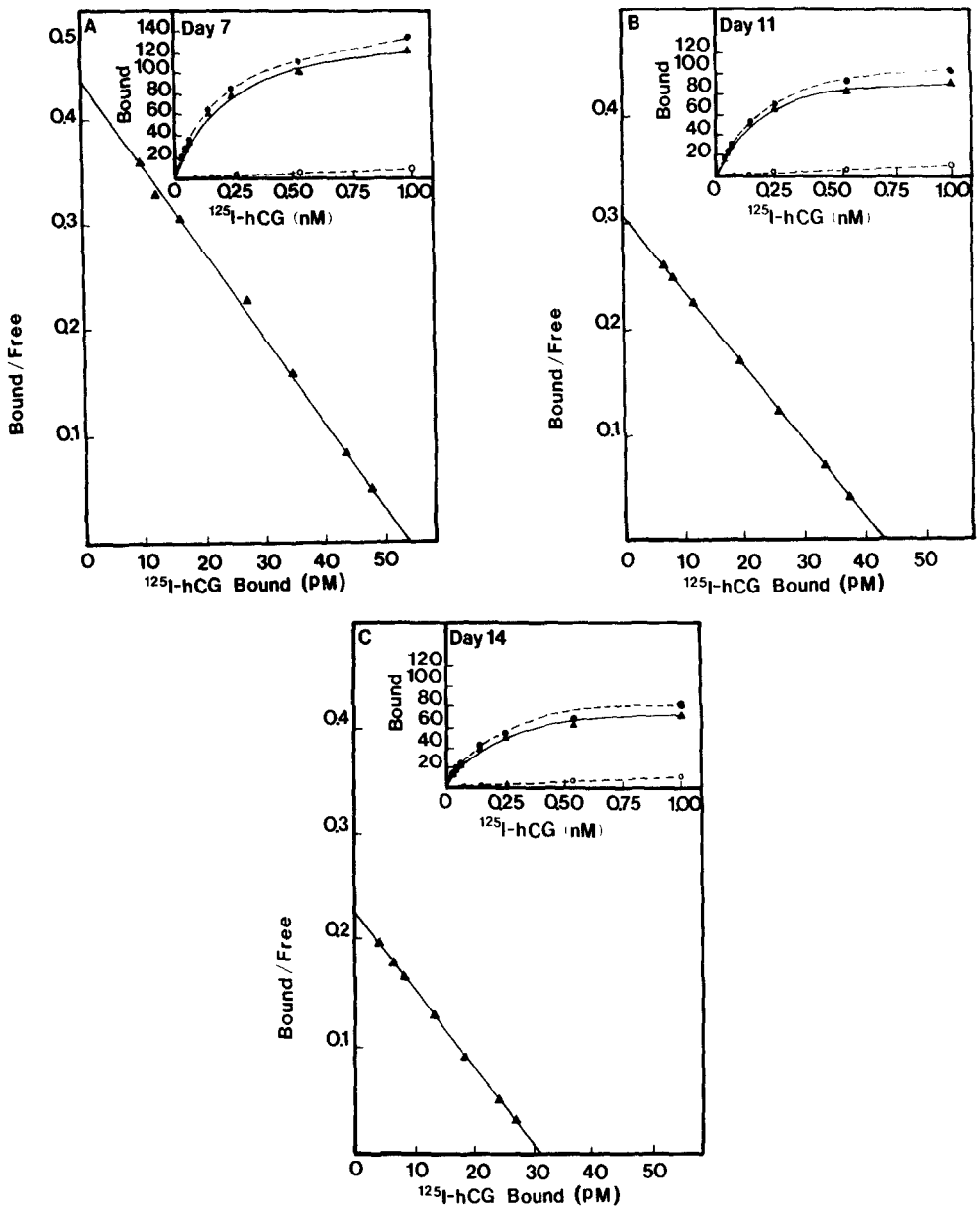

Fig. 2. Scatchard analysis of the binding of ^{125}I-hCG to the membranes obtained from day 7, 11 and 14 of pseudopregnant ovary.

Rats were sacrificed on days 7, 11 and 14 after hCG injection and plasma membranes were prepared from the isolated ovaries. Aliquots of membranes (125 µg membrane protein) were incubated with various concentrations of ^{125}I-hCG from 0.05 nM to 1.00 nM in a total volume of 0.3 ml binding assay buffer (total binding). Incubation was carried out at 37°C for 60 min. For estimation of nonspecific binding, unlabeled hCG was added at a final concentration of 1.5 µM. The specific binding was calculated by subtracting the nonspecific binding from the total binding. The insets show the binding data. The binding of ^{125}I-hCG as a function of ligand concentration was then transformed into Scatchard plot (7). The ^{125}I-hCG binding activity is expressed as fmole per mg membrane protein.

●, total binding; O, nonspecific binding; ▲, specific binding

Table I

The ^{125}I-hCG binding affinity and binding capacity of the plasma membranes obtained from day 7, 11 and 14 of pseudopregnancy

	Kd (M)	Binding Capacity (fmol/mg membrane protein)
Day 7	1.25×10^{-10}	130
Day 11	1.42×10^{-10}	104
Day 14	1.37×10^{-10}	74

The data were derived from Fig. 2.

(9). The results presented in Fig. 3 show that all four subunits of the LH/hCG receptor were detected in each cell preparation, while the intensity of the ^{125}I-hCG linked receptor subunits decreased parallel to the decrease in hCG binding observed on day 7 to day 14. These results further suggest

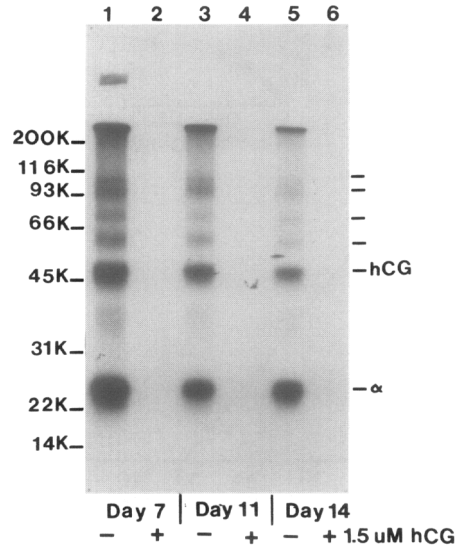

Fig. 3 Examination of the subunit structure of the LH/hCG receptor during different time periods of pseudopregnancy.

Rat luteal cells obtained from day 7, 11 and 14 of pseudopregnancy were equilibrated with 0.5 nM ^{125}I-hCG at 37°C for 2 h. Incubation was terminated by the addition of 1 ml of ice-cold Tris buffer, pH 7.4, followed by centrifugation at 3,000 x g for 10 min to remove unbound ^{125}I-hCG. The ^{125}I-hCG-equilibrated luteal cells were cross-linked with 1 mM DSS at 4°C for 15 min. These cells were solubilized in sample buffer containing 2% SDS and 2% β-mercaptoethanol, boiled for 5 min in a water bath and then electrophoresed (10) in a 0.1% SDS, 10% acrylamide slab gel under reducing conditions. The gel was dried before autoradiography. The migration distance of the molecular weight markers is shown to the left of the autoradiogram.

that the decrease in ^{125}I-hCG binding activity in pseudopregnant rat ovarian membranes from day 7 to day 14 is due to a decrease in receptor concentration rather than a change in receptor subunit structure.

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