NOTE
In Vitro Examination of LH-HCG Receptors in Human Corpora Lutea of the Menstrual Cycle and Pregnancy

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Abstract

Binding of 125I-human chorionic gonadotropin (HCG) to homogenates of the human corpora lutea of the menstrual cycle and pregnancy was examined in vitro. While corpora lutea of the menstrual cycle bound 125I-HCG specifically, the corpora lutea of pregnancy from 8 weeks' gestation bound little or none of the added 125I-HCG. In the corpus luteum of the menstrual cycle the dissociation constant for HCG and the number of binding sites were analyzed by a Scatchard plot. However, from these observations along with the findings of earlier reports, it may be suggested that LH-HCG receptors remain reduced in number in the corpus luteum of pregnancy.

It is generally recognized that peptide hormones exert their primary actions on target cells by binding to specific receptor sites in the plasma membrane. The existence of receptors for luteinizing hormone (LH) and human chorionic gonadotropin (HCG) has been well documented in corpora lutea of various species. Therefore, it may be accepted that the stimulatory effect of HCG upon human corpus luteum of the menstrual cycle was demonstrated in vivo (Hanson et al., 1971) and in vitro (Marsh and LeMaire, 1974) in terms of steroidogenesis and cyclic adenosine 3', 5'-monophosphate (c-AMP) accumulation. However, in contrast, it was reported that steroidogenesis in corpus luteum of pregnancy could not be enhanced by HCG administration in vivo (Runnebaum et al., 1972; Tu, 1978) and in vitro (Nakashima, 1979). The present study was designed to elucidate whether or not LH-HCG receptor existed in human corpus luteum of pregnancy.

Materials and Methods

Six corpora lutea of the menstrual cycle were obtained in operations performed for uterine myoma and benign ovarian tumor, while 11 corpora lutea of pregnancy (7th week of gestation, 2; 8th, 3; 10th, 5; 12th, 1), were obtained in operations for tubal sterilization with medical termination and ectopic pregnancy. They were stored at -80°C until processed. The days of both the menstrual cycle and pregnancy were estimated from the patients' menstrual history with or without endometrial histology.

Corpora lutea were thawed, dissected clean from adjacent tissues, weighed, and homogenized with Teflon homogenizer at 1,000 rpm for 10 strokes in 10 mM Tris-HCl buffer of pH 7.4, containing 250 mM sucrose, 10 mM Mg++, and Ca++, 50 mM Na+ and 0.2% (w/v) bovine serum albumin (BSA). The homogenate was centrifuged at 2,000 x g for 15 min. The protein of the supernatant was measured by the method of Lowry et al. (1951).

Five μg of highly purified HCG (10,000 to 12,000 IU/mg) (Okumura et al., 1976) was added to 1 mCi of Na125I (purchased from New England Nuclear Corp., USA), followed by 5 μg of chloramine-T.
After 1 min of incubation at room temperature 20 μg of sodium metabisulfite was added. Labeled HCG was purified by gel filtration on Sephadex G-50 column (0.8 × 15 cm). Specific activity of labeled HCG was about 50 μCi/μg. Labeled HCG has been shown to retain full biological activity by the radioreceptor assay system for HCG using rat testicular homogenate (Tanabe, 1978). Newly labeled HCG was used at analysis.

The mixture of 200 to 400 μl aliquots of the supernatant and 50,000 to 200,000 cpm of 125I-HCG with or without 10 IU unlabeled HCG (4,190 IU/mg) was incubated at 37°C for 6 hr. Bound and free 125I-HCG were separated by centrifugation. The radioactivities of the sediment were counted with an Aloka Autogamma spectrometer (Aloka Co., Tokyo) with 125I efficiency of 60%. The results were analyzed by Scatchard plot (Scatchard, 1949).

Results

Figure 1 illustrates the radioactivity of the pellets in the absence and presence of cold HCG (10 IU) in 8 out of 17 human corpora lutea examined. Two corpora lutea of the menstrual cycle showed about 16,000 cpm in the absence of cold HCG, while the bound cpm was found markedly reduced in the presence of cold HCG (about 2,700 cpm). In corpora lutea at the 8th and 10th weeks of gestation no reduction of the bound cpm was observed if an excess of unlabeled HCG was added. The bound cpm was found to be slightly, but not significantly, reduced in corpora lutea at the 7th week.

Figure 2 shows the Scatchard plot for HCG receptor in one corpus luteum of the menstrual cycle, assuming the molecular weight of HCG as 36,700 (Birken and Canfield, 1978). The plot indicated two different receptors: one possessing the characteristics of high affinity and low capacity; the other, low affinity and high capacity. In the former of these receptors the number of binding sites was 8.41 pm/mg protein and the dissociation constant (Kd) was $9.58 \times 10^{-10}$ M. In the latter the number was 94.1 pm/mg-protein and Kd was $4.71 \times 10^{-8}$ M.

Discussion

The present study showed that corpus luteum of the menstrual cycle contained specific HCG receptor, while HCG receptor could not be detected in the corpus luteum after the 8th week of gestation. These data are in agreement with those of Cole et al. (1973) and of Rao et al. (1977). These results appear to support the data showing
that intramuscularly or intravenously administered HCG of 5,000 to 100,000 IU resulted in no marked changes in human serum progesterone concentration during early pregnancy (Runnebaum et al., 1972; Tu, 1978), and also that no remarkable increase in steroidogenesis and cAMP accumulation could be observed following the addition of 100 IU/ml HCG in the incubation experiments of corpora lutea of pregnancy (Nakashima, 1979). Furthermore, the above observations correlate well with the finding that human corpus luteum was not needed for the maintenance of pregnancy after the 7th week of gestation (Csapo et al., 1972).

In corpus luteum of pregnancy it was assumed that (1) receptors were present, but they were saturated with endogenous HCG, or (2) there were few or no receptors (Rao et al., 1977). In order to test the first possibility, some experiments were performed to dissociate and remove endogenous HCG by shifting the pH of the incubation medium from 7.2 to 10.0 (Wardlaw et al., 1975) and from 7.0 to 2 (Rao et al., 1977). In our laboratory, attempts were also made by means of the technique mentioned above and by pre-treatment of the supernatant with a very sensitive and specific anti-HCG antibody (unpublished data). None of the trials succeeded in recovering LH-HCG receptors from the corpus luteum of pregnancy.

In addition, from the viewpoint of receptor control mechanism, the fact of a decrease in insulin receptor concentrations produced by chronic exposure of cultured human lymphocytes to insulin (Gavin et al., 1974), seems to indicate that the number of LH-HCG receptors may decrease as serum HCG levels drop rapidly during early pregnancy. This supposition appears to be supported by the existence of a few LH-HCG receptors in the corpus luteum of the 7th week and also by the finding that the infusion of HCG maintained the corpus luteum function after delivery (LeMaire et al., 1971). The correlation between serum HCG levels and the number of LH-HCG receptors in corpora lutea, however, must be further examined prior to the 7th week, though it could not be demonstrated in this report since it is very difficult to obtain human corpora lutea at such early stages.

Although the present data showed that LH-HCG receptors were undetectable in the corpora lutea of pregnancy, it might be said that the corpus luteum of pregnancy still remains a functioning endocrine organ as shown in the findings that sex steroids (Mikhail ans Allen, 1967) and 3β-hydroxysteroid dehydrogenase activity (Strauss et
were determined. Provided that there were no receptor for LH-HCG, human corpus luteum of pregnancy might need no luteotropic hormone, or other hormone(s) might play an important role in the maintenance of the corpus luteum.

From these considerations we concluded that LH-HCG receptors might be present in the human corpus luteum of pregnancy, but that they might be few in number, unable to be detected by means of the traditional techniques.

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References


