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Identification of LH/hCG Receptors in Rabbit Uterus (42775)

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Abstract. Luteinizing hormone (LH) is believed to act via specific receptors to control gonadal steroidogenesis and reproductive processes. Recently A. J. Ziecik, P. D. Stanchev, and J. E. Tilton (Endocrinology 119:1159, 1986) reported surprisingly that LH/hCG receptors were present in porcine uterus, a tissue not known to be a target for LH action. We report herein the identification of high-affinity LH receptors in the rabbit uterus. Uteri from adult New Zealand white rabbits were homogenized in Tris–HCl, 0.25 *M* sucrose. After filteration and sequential centrifugation, a partially purified pellet containing receptors was obtained. This preparation was incubated with a trace (1300 cpm) (50 pg) ^{125}I-labeled chorionic gonadotropin and with various unlabeled protein hormones. Receptor bound was separated from free hormone by centrifugation at 1000*g*. Affinity was estimated by Woolf plot analysis. Specific binding sites for LH/hCG were identified. The following K_d's were calculated: human LH, 1.6×10^{-11}; hCG, 0.5×10^{-11}; human TSH, 1.3×10^{-9}; and human FSH, 7.85×10^{-9}. The reaction of human FSH and TSH with the receptor is best explained by LH contamination of these hormones. A similar preparation of rat liver showed that no binding sites were present. Rabbit ovarian LH receptors had a K_d slightly higher at 4.1×10^{-11} than that of the uterine LH receptors. Rabbit ovarian receptors were present at 2.27×10^{-13} *M*/mg protein compared to uterine receptors at 4.65×10^{-15} *M*/mg protein. We conclude specific- and high-affinity binding sites (receptors) for LH are present in the rabbit uterus. The function of these receptors remains unknown. © 1988 Society for Experimental Biology and Medicine.

Luteinizing hormone (LH) is a glycoprotein hormone synthesized and secreted by specialized cells in the anterior pituitary. LH and a second pituitary gonadotropin, follicle-stimulating hormone (FSH), together control gonadal steroid secretion and gamete development in both males and females.

Recently Ziecik *et al.* (1) reported that the porcine uterus contained high-affinity receptors for human chorionic gonadotropin (hCG), an "LH-like" hormone. Since the uterus is not known to be a target tissue for LH or hCG, this report was surprising. For over 10 years we have employed rabbits in studies of reproductive endocrinology. In order for us to further study LH/hCG binding to uterus, the present study was designed to determine if there are LH/hCG receptors in rabbit uteri and to compare the binding affinity and specificity of these receptors to those found in the ovary.

Materials and Methods. *Hormone preparations.* Most of the hormonal preparations used in these studies were kindly supplied by the hormone distribution program of NIDDK. Human chorionic gonadotropin (hCG) from two sources was used: (i) a crude commercial hCG (Organon 500 IU/vial), and (ii) highly purified hCG CR-121 kindly supplied by NICHD. For purposes of estimating K_d, we assumed the hCG in the impure commercial preparation had a potency of 10,000 IU/mg. The purified luteinizing hormone used was human LH (hLH) 6332-B. The purified follicle-stimulating hormones used were human FSH AFP5884-B and rabbit FSH (AFP-538-6); the purified thyrotropin (TSH) was human TSH AFP 4197-C.

Preparation of membrane fraction. For each of five independent experiments, four adult female New Zealand white rabbits were sacrificed by intravenous air embolus. Using tissues from four animals complete receptor analysis for three hormones was performed. All experiments were repeated at least twice on another occasion. Uterus, liver, and ovaries were removed at sacrifice and were minced with scissors and weighed. The cell membrane fractions from each tissue speci-

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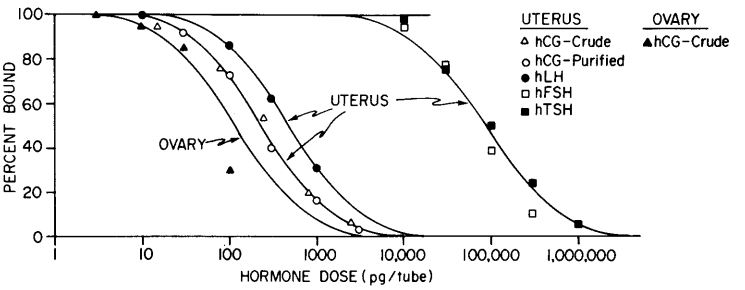

FIG. 1. Dose response curves for various hormones using uterine or ovarian receptor preparations.

men were obtained by following the procedure modified from Ziecik *et al.* (1). Each tissue was homogenized at 4°C in a 6-vol (vol/wt) buffer (25 m*M* Tris–HCl, ph 7.4, containing 0.25 *M* sucrose) until liquified. The homogenate was filtered through one layer of gauze, and the filtrate was centrifuged for 20 min at 800*g*. The resulting supernatant was suspended in 3 ml ice-cold 25 m*M* Tris–HCl, pH 7.2, containing 0.1% BSA and 5 m*M* $MgCl_2$. This suspension was stored at −70°C until used.

Measurement of LH/hCG receptor dissociation constant. hCG was labeled using the chloramine T method as modified in laboratory (2). Specific activity of the labeled hCG averaged 25 μCi/μg. The receptor assay tube included the following: (i) 0.1 ml 25 m*M* Tris–HCl (pH 7.2) containing 5 m*M* $MgCl_2$ and 0.1% BSA (buffer), (ii) 0.1 ml buffer containing the hormone being studied, (iii) 0.1 ml containing 1300 cpm ^{125}I-labeled hCG (approximately 50 pg; note this is a 10-fold smaller amount than reported by Ziecik *et al.* (1)), and (iv) 0.2 ml receptor preparation. The assays incubated overnight at room temperature. Receptor bound was separated from free hormone by centrifugation at 2000*g* for 20 min. The supernatant was removed by aspiration and the cpm determined in the pellet in an automatic gamma spectrometer.

Nonspecific binding was determined in the presence of excess unlabeled hCG (10 IU/tube) and was <6%. The dissociation constants (K_d) were determined using the Woolf plot analysis (3). Kneightley and Cressie (4) have presented data to indicate the Woolf plot has some advantages over the more commonly used Scatchard plot (5). For the determination made in this study, the Woolf plot and Scatchard plot analysis gave similar results.

Results. ^{125}I-hCG bound to the membrane fraction of the uterus and unlabeled hCG and LH competitively displaced bound hormone. Figure 1 shows the dose response curves for hLH, hFSH, hCG, and hTSH using ^{125}I-hCG as the labeled analyte. hLH and the two hCG preparations were potent in competitively displacing labeled hCG binding. hFSH and hTSH also showed reaction in this assay system but much greater quantities were required to displace the assay tracer. A membrane fraction prepared in identical fashion from rabbit liver showed no specific binding for LH/hCG.

Figure 2 shows the Woolf plots of these data. From these data the K_d's shown in Table I were calculated. In addition, the K_d calculated for hFSH was 7.85×10^{-9} and for hTSH 1.3×10^{-9}. As indicated, the reaction

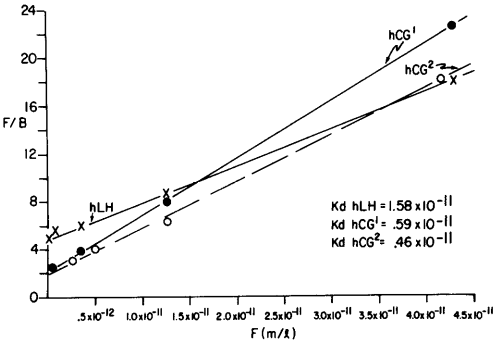

FIG. 2. Woolf plot of hLH and hCG binding to uterine receptor preparations. Each point represents the average of three replicates. hCG^1 is a crude preparation from commercial sources. hCG^2 is purified hCG CR-121.

TABLE I. DISSOCIATIONS CONSTANTS (K_d) OF RABBIT LH/hCG RECEPTORS

Hormone	Tissue	K_d	95% Limits
hLH	Uterus	1.58×10^{-11}	$(1.32\text{–}1.84 \times 10^{-11})$
hCG[1]	Uterus	0.59×10^{-11}	$(0.50\text{–}0.67 \times 10^{-11})$
hCG[2]	Uterus	$.46 \times 10^{-11}$	$(0.34\text{–}0.57 \times 10^{-11})$
hCG[2]	Ovary	4.05×10^{-11}	$(3.79\text{–}4.32 \times 10^{-11})$

Note. hLH, human luteinizing hormone; hCG[1], purified human chorionic gonadotropin (CR-121); hCG[2], crude hCG. K_d for each hormone was calculated from two independent experiments. Tissues from 20 animals and five experiments were required for these studies.

of both hFSH and hTSH is best explained by contamination of these preparations with hLH. The number of LH/hCG binding sites was also calculated. The uterus contained an average of 4.65×10^{-15} M/mg protein; the ovary contained 2.27×10^{-13} M/mg protein.

Discussion. These studies demonstrate that high-affinity hCG/hLH receptors exist in the rabbit uterus, a tissue not known to be a target organ for hCG/hLH. hFSH and hTSH also showed reaction with these receptors, but much greater quantities were required to displace the assay tracer. The small reactions are compatible with the contamination of TSH and FSH with LH as described in the NIAMDD inserts accompanying the hormone. According to the specifications from NIDDK, this hFSH preparation contains 4.5% hLH (595 IU/mg by RIA), while the hTSH contains 7.6% hLH (995 IU/mg by RIA). Ziecik *et al.* (1) used about 10 times the amount of tracer we used (50 pg versus 500 pg). We found that use of this smaller amount of tracer was required in rabbit uterus to demonstrate specific binding. As may be seen in Fig. 1, 500 pg of hCG produced markedly decreased specific binding of tracer. However, our data support the report of Ziecik *et al.* (1) who reported hCG/hLH receptors in the porcine uterus. Interestingly, they found such receptors in all preparations of myometrium and about 30% of membrane fraction of endometrium. The K_a they reported (10^{11}) ($K_d = 1/K_a$) was similar to those we found. Presumably, these receptors bind rabbit LH with high affinity, but rabbit LH has not been studied. The K_d of these binding sites is greater for hLH binding than for hCG binding. hCG binding to ovarian receptors has a higher K_d than hCG binding to uterine receptors. In addition, the ovary contained approximately 50 times greater number of receptors per milligram protein than did the uterus. The function (if any) of these high-affinity uterine receptors is uncertain. Johnson *et al.* (6, 7) have shown that ACTH binds with high affinity to mouse splenocytes, a cell not previously known to be an ACTH target. Further, they showed ACTH inhibited production of antibodies and of interferon by such cells. It is reasonable to conjecture that LH has an action on uteri as yet undefined. Since we have employed the rabbit as an experimental animal in a large number of studies in reproductive endocrinology during the past 10 years, the demonstration of hCG/LH receptors in rabbit uterus is an important first step in elucidating function.

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