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ACTIVATION BY HUMAN CHORIONIC GONADOTROPIN OF OVARIAN CARBONYL REDUCTASE IN MATURE RATS EXPOSED *IN VIVO* TO ESTROGENS

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Abstract—We investigated the effects of exogenous estrogens and human chorionic gonadotropin (hCG) on the activity, content, and immunohistochemical localization of ovarian carbonyl reductase (CR) in mature cycling rats. Estrogens, estradiol, hexestrol (HEX) and diethylstilbestrol (DES) were given s.c. to rats daily for 3 days from the first day of diestrus, and hCG was given s.c. at 3:00 p.m. on the day of expected proestrus. The ovaries were isolated on the day of expected estrus. Ovarian CR activity was measured by using two substrates that reflect the activity of the enzyme in rats, and the enzyme content was determined by western blot analysis. Ovarian CR activity and content were decreased by estrogens as well as by inhibition of ovulation; hCG restored both the activity and the content decreased by estrogens to levels produced by hCG alone. Nevertheless, the number of ova in the oviduct when ovulation was decreased or blocked by estrogens was not restored completely by hCG treatment. Faint immunostaining in the interstitial gland cells of HEX-treated rat ovaries was observed. These results suggest that (i) although hCG activates ovarian CR in estrogen-treated rats, this increase in both enzyme activity and content may not be an obligatory event in the ovulatory process, and (ii) exogenous estrogens may predominantly influence the ovarian CR in the interstitial gland cells in mature rats by inhibiting luteinizing hormone release from the pituitary.

Key words: carbonyl reductase; rat ovary; estrogen; hCG; immunochemistry

CR§ (EC 1.1.1.184), also known as PG 9-ketoreductase, is a soluble, monomeric oxidoreductase that catalyzes the NADPH-dependent reduction of endogenous and xenobiotic carbonyl compounds such as PGs, steroids and quinones [1]. CRs have been isolated from a number of species and tissues, including human placenta [2], brain [3], liver [4] and testis [5], as well as rat ovary [6] and testis [7]. We and others have reported that two isoforms of CR (CR1 and CR2) from rat ovary NADPH-dependently catalyze the reduction of PGE_2 to $PGF_{2\alpha}$ and reduce the 15-keto group of 15KD-$PGF_{2\alpha}$, which has no bioactivity, to $13,14H_2$-$PGF_{2\alpha}$, which is a bioactive PG [6, 8, 9].

We have demonstrated that ovarian CR activity and its content are increased markedly following an increase in estrogen-induced LH on the evening of proestrus in mature rats [10], and that the enzyme activity is decreased rapidly by day 14 of pregnancy in rats, whereas the enzyme content maintains a constant level by day 18 of pregnancy [11]. These results suggest that ovarian CR, which possesses PG-metabolizing activity, is closely involved not only in the ovulatory process but also in luteal functions. Indeed, in immature rats, the positive immunoreactivity with anti-ovarian CR antibody is observed in theca interna cells and interstitial gland cells at 72 hr after PMSG treatment when ovulation is confirmed, but not at 48 hr [12].

The increase in ovarian weight and follicular growth is regulated by the hypothalamo-pituitary-ovarian axis, and it is well accepted that the endogenous estrogen may play an important role in the ovary [13, 14]. Chemical antiestrogen inhibits ovulation as well as ovarian CR activity in mature rats, whereas hCG and LH-RH completely restore these activities to control levels [15, 16]. This result indicates that antiestrogen prevents endogenous estrogen from expressing its full effects on estrogen target tissues, and that both endogenous estrogen and gonadotropins are indispensable for the expression of ovarian CR. Exogenous estrogen is well known to stimulate a series of events, such as DNA synthesis and cell division, in ovariectomized rat uterus. On the other hand, Iguchi *et al.* [17] have reported that exogenous estrogen, DES, directly induces polyovular follicles in the mouse ovary. Abnormalities of the oviduct and ovary have been reported in rats and mice exposed to DES [18, 19]. However, the effect of exogenous estrogen on rat ovarian CR activity and its content is not known.

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§ Abbreviations: CR, carbonyl reductase; PG, prostaglandin; 15KD-$PGF_{2\alpha}$, 13,14-dihydro-15-keto-$PGF_{2\alpha}$; $13,14H_2$-$PGF_{2\alpha}$, 13,14-dihydro-$PGF_{2\alpha}$; LH, luteinizing hormone; PMSG, pregnant mare serum gonadotropin; hCG, human chorionic gonadotropin; PAP, peroxidase anti-peroxidase; LH-RH, luteinizing hormone-releasing hormone; DES, diethylstilbestrol; HEX, hexestrol; 4BP, 4-benzoylpyridine; and DTT, dithiothreitol.

The present study was undertaken to determine the effects of exogenous estrogens, estradiol and synthetic estrogens, and the effect of hCG following exposure to estrogens on changes in ovarian CR in mature cycling rats.

MATERIALS AND METHODS

Animals. Seven-week-old female Wistar-KY rats were purchased from the Shizuoka Laboratory Animal Center (Shizuoka, Japan), and were housed in group cages (4 or 5 rats per cage) under controlled conditions of light (12 hr on, 12 hr off) and temperature (24°). Water and food were always available. Only animals that had shown at least two normal 4-day cycles, as determined by daily vaginal smears immediately before the start of the experiment, were used.

Chemicals. NADPH was obtained from the Oriental Yeast Co. (Osaka, Japan). Estradiol, HEX and DES were purchased from the Sigma Chemical Co. (St. Louis, MO, USA), and hCG was obtained from Teikoku Hormone Mfg. (Tokyo, Japan). 4BP, DTT and EDTA were obtained from the Wako Pure Chemical Co. (Osaka, Japan). [5,6,8,9,11,12,14-^{3}H]-15KD-$PGF_{2\alpha}$ (sp. act. 80 Ci/mmol) was purchased from Amersham Int. plc. (Buckinghamshire, U.K.) and authentic 15KD-$PGF_{2\alpha}$ from Upjohn Pharmaceuticals Ltd. (Kalamazoo, MI, USA). Authentic 13,14H_2-$PGF_{2\alpha}$ was provided by the Ono Pharmaceutical Co. (Osaka, Japan). Other chemicals of reagent grade were obtained from the Wako Pure Chemical Co. and the Bio-Rad Co. (Tokyo, Japan).

Hormone treatment. Estradiol, HEX and DES were dissolved in sesame oil, and hCG was dissolved in 0.9% saline solution. Estradiol (1, 10 and 100 μg/rat) and synthetic estrogens (0.2 mg/rat) were administered s.c. to rats daily for 3 days from the first day of diestrus, and hCG (25 IU/rat) was administered s.c. at 3:00 p.m. on the day of expected proestrus. Control animals were given (s.c.) vehicle alone. The ovaries of each rat were isolated at 9:00 a.m. on the day of expected estrus, and homogenized in 8 mL of ice-cold 10 mM phosphate buffer (pH 6.5) containing 0.154 M KCl, 1 mM DTT and 0.5 mM EDTA. The presence of ova in the oviduct was determined microscopically [10, 16].

Enzyme assay. The ovarian homogenate was centrifuged at 4° for 60 min at 105,000 *g* by a Hitachi Automatic Centrifuge model 70P-72, and the 105,000 *g* supernatant (cytosolic fraction) obtained was used as a crude enzyme preparation for the assay of enzyme activity. The reduction of 4BP (1 mM) was assayed in 1 mL of incubation mixture, consisting of 100 mM phosphate buffer (pH 6.5), ovarian cytosol, substrate solution and NADPH (final concentration 0.1 mM), for 3 min at 37° by a Hitachi 150-20 Spectrophotometer. One unit of enzyme activity was expressed as the amount of enzyme that oxidized 1 μmol of NADPH/min at 340 nm under the assay conditions. 15KD-$PGF_{2\alpha}$ reducing activity was determined by a radiochemical method as previously described [20] and expressed as picomoles per milligram of protein per 15 min of 13,14H_2-$PGF_{2\alpha}$ formed. Protein concentration in the ovarian cytosol was determined by the method of Lowry *et al.* [21].

Enzyme quantitation. The quantitation of CR in rat ovarian cytosol was performed by western blot-PAP analysis using anti-rat ovarian CR, CR2, antiserum that was raised against the purified rat ovarian CR [6] as previously described [22]. Briefly, ovarian cytosolic protein was subjected to SDS-PAGE (10% gel) according to the method of Laemmli [23] and blotted onto nitrocellulose membrane (BA83, 0.2 μm) obtained from the Schleicher & Schuell Co. (Dassel, Germany). Enzyme protein on the membrane was visualized by the method of Hosokawa *et al.* [24], and the amount of the enzyme protein was measured by a densitometer using purified rat ovarian CR, CR2, as a standard.

Immunohistochemical methods. The ovaries were isolated quickly, immersed immediately in 100 mM phosphate-buffered (pH 7.4) 4% paraformaldehyde, and fixed for 6 hr. After fixation, the samples were rinsed in 100 mM phosphate buffer (pH 7.4), sequentially dehydrated in 70–100% alcohol, and embedded in paraffin. The paraffin blocks were cut into 4-μm thick slices, and the immunostaining with anti-rat ovarian CR, CR2, antiserum was carried out by the avidin-biotin peroxidase complex (ABC) method with a Vectastain ABC kit (Vector Lab., CA) as previously described [10–12].

Statistical analysis. All results are expressed as means ± SEM. Duncan's multiple range test was used for comparison between groups, with a P value of 0.05 or less considered to indicate a significant difference.

RESULTS

Effects of estradiol and hCG. Changes in body weight, ovarian weight and ovarian CR content in relation to ovulation after treatment with estradiol and hCG are summarized in Table 1. No significant decrease in body weight was observed, even in a group of rats given 100 μg estradiol for 3 days. hCG significantly increased ovarian weight as compared with control (estrus), but not ovarian CR content or the number of ova in the oviduct. A significant decrease in ovarian weight was observed only at a dose of 100 μg estradiol; ovulation was also completely inhibited at this dose. Lower doses (1 and 10 μg) of estradiol affected both the number of rats ovulating and the number of ova; that is, 1 μg of estradiol inhibited ovulation in 2 of 8 rats and 10 μg of estradiol in 4 of 10 rats. Furthermore, 10 μg of estradiol significantly decreased the number of ova in ovulated rats, whereas the number of ova in ovulated rats administered 1 μg of estradiol did not change. The administration of hCG to estradiol-treated rats increased ovarian weight to levels obtained with hCG alone, and the number of ovulated rats was restored, although the number of ovulated rats in the group treated with 100 μg of estradiol was 3 out of 4. However, hCG did not completely restore the number of ova in rats treated with 10 and 100 μg of estradiol (10 ± 0.6 and 4 ± 1.9, respectively). On the other hand, no significant effect of hCG was seen on ovarian CR content in estradiol-treated rats, although the enzyme content

Table 1. Effects of estradiol and hCG on body weight, ovarian weight and ovarian CR content in rats in relation to ovulation

	Body wt (g)	Ovarian wt (mg)	No. of ova (ovulated rats)	CR content (μg/mg protein)
Control (5)	204.1 ± 4.36	74.5 ± 0.77	12 ± 0.1 (5/5)	24.46 ± 3.466
hCG (5)	199.1 ± 5.07	83.7 ± 1.85*	11 ± 0.6 (5/5)	43.64 ± 8.300
Estradiol				
1 μg (8)	206.3 ± 2.78	74.8 ± 1.48	12 ± 0.7 (6/8)	21.75 ± 3.765
+hCG (4)	210.5 ± 2.25	86.0 ± 1.08*†	12 ± 0.6 (4/4)	37.70 ± 7.329
10 μg (10)	194.1 ± 7.25	68.6 ± 3.70	9 ± 0.9* (6/10)	16.83 ± 7.062
+hCG (4)	196.4 ± 5.68	84.3 ± 4.99‡	10 ± 0.6§ (4/4)	27.62 ± 1.587
100 μg (4)	195.8 ± 3.62	66.2 ± 1.23*	0 (0/4)	14.46 ± 4.501
+hCG (4)	198.8 ± 4.09	85.3 ± 1.03*†	4 ± 1.9* (3/4)	26.37 ± 3.128

Estradiol was given s.c. to rats for 3 days from the first day of diestrus, and hCG was given s.c. at 3:00 p.m. on the day of expected proestrus. Each value represents the mean ± SEM.
* Significantly different from control (estrus), $P < 0.01$.
†‡ Significantly different from estradiol alone: † $P < 0.01$, and ‡$P < 0.05$.
§ Significantly different from control (estrus), $P < 0.05$.

Table 2. Changes in ovarian CR activity towards two substrates in rats after treatment with estradiol and hCG

	CR activity	
	15KD-$PGF_{2\alpha}$ (pmol/mg protein)	4-Benzoylpyridine (units × 10^{-3}/mg protein)
Control (5)	312.49 ± 30.455	16.03 ± 0.857
hCG (5)	362.17 ± 34.119	18.87 ± 1.831
Estradiol		
1 μg (8)	281.86 ± 22.190*	14.05 ± 1.630
+hCG (4)	350.25 ± 15.792*	21.32 ± 1.534†‡
10 μg (10)	230.97 ± 40.021	12.41 ± 2.236
+hCG (4)	379.42 ± 23.894†	18.12 ± 0.967†
100 μg (4)	207.57 ± 13.015‡	11.48 ± 0.616§
+hCG (4)	403.44 ± 28.724*	20.90 ± 0.602*§

The experimental conditions are the same as described in the legend of Table 1. Each value represents the mean ± SEM.
*† Significantly different from estradiol alone: * $P < 0.01$, and † $P < 0.05$.
‡§ Significantly different from control (estrus): ‡ $P < 0.05$, and § $P < 0.01$.

was on the upward trend as compared with estradiol alone.

Table 2 shows changes in ovarian CR activity towards two substrates that reflect ovarian CR activity in rats [6, 10, 11], after treatment with estradiol and hCG. Both 15KD-$PGF_{2\alpha}$ and 4BP reducing activities were decreased in a dose-dependent manner with estradiol, and 100 μg estradiol significantly decreased both of the enzyme activities to 66.4% and 71.6%, respectively, of control (estrus) levels. Subsequent administration of hCG to estradiol-treated rats restored both 15KD-$PGF_{2\alpha}$ and 4BP reducing activities to the levels of hCG alone, and caused a 1.9- and 1.8-fold increase, respectively, in both activities, which had been suppressed by 100 μg estradiol.

Effects of synthetic estrogens and hCG. Table 3 summarizes changes in body weight, ovarian weight and ovarian CR content in relation to ovulation after treatment with two synthetic estrogens (0.2 mg/rat each) and hCG. Significant decreases in body weight and ovarian weight were observed by treatment with either HEX or DES. Ovulation was not seen in rats treated with either HEX or DES. Similarly, ovarian

Table 3. Effects of synthetic estrogen on body weight, ovarian weight and ovarian CR content in rats in relation to ovulation

	Body wt (g)	Ovarian wt (mg)	No. of ova (ovulated rats)	CR content (μg/mg protein)
Control (5)	206.4 ± 5.16	74.8 ± 1.71	12 ± 0.2 (5/5)	19.23 ± 1.822
hCG (10)	205.0 ± 3.83	85.9 ± 2.04*	11 ± 0.6 (10/10)	30.82 ± 3.904*
Hexestrol				
0.2 mg (8)	162.9 ± 2.82*	64.1 ± 2.20*	0 (0/8)	15.32 ± 1.638
+hCG (6)	149.8 ± 4.71*†	90.7 ± 3.36*‡	9 ± 1.3*§ (6/6)	32.44 ± 1.753*‡
Diethylstilbestrol				
0.2 mg (9)	156.7 ± 2.37*	62.4 ± 1.73*	0 (0/9)	10.54 ± 1.088*
+hCG (4)	158.3 ± 2.39*	84.3 ± 1.44*‡	7 ± 0.8* (4/4)	29.06 ± 1.753*‡

Each synthetic estrogen was given s.c. to rats for 3 days from the first day of diestrus, and hCG was given s.c. at 3:00 p.m. on the day of expected proestrus. Each value represents the mean ± SEM.
*Significantly different from control (estrus), $P < 0.01$.
†‡ Significantly different from synthetic estrogen alone: † $P < 0.05$, and ‡ $P < 0.01$.
§ Significantly different from control (estrus), $P < 0.05$.

Table 4. Changes in ovarian CR activity towards two substrates in rats after treatment with synthetic estrogen and hCG

	CR activity	
	15KD-$PGF_{2\alpha}$ (pmol/mg protein)	4-Benzoylpyridine (units × 10^{-3}/mg protein)
Control (5)	292.01 ± 17.349	13.56 ± 0.488
hCG (10)	306.26 ± 12.923	16.43 ± 0.806*
Hexestrol		
0.2 mg (8)	204.03 ± 20.806†	9.11 ± 1.811*
+hCG (6)	333.63 ± 16.873‡	14.37 ± 0.416§
Diethylstilbestrol		
0.2 mg (9)	204.98 ± 24.127*	10.16 ± 0.629†
+hCG (4)	323.00 ± 11.230‡	18.30 ± 0.579†‡

The experimental conditions are the same as described in the legend of Table 3. Each value represents the mean ± SEM.
*† Significantly different from control (estrus): *$P < 0.05$, and † $P < 0.01$.
‡§ Significantly different from estrogen alone: ‡$P < 0.01$, and § $P < 0.05$.

CR content was decreased by both estrogens, although no significant difference was observed between the control and the HEX-treated groups. hCG increased not only ovarian weight but also ovarian CR content as compared with the controls, and restored ovarian weight and ovarian CR content in rats treated with either HEX or DES to the levels of hCG alone. However, the number of ova in rats treated with either estrogen was not restored to control levels by hCG treatment, although ovulation was observed in all rats treated with hCG.

Table 4 shows changes in ovarian CR activity towards two substrates after treatment with synthetic estrogens and hCG. 15KD-$PGF_{2\alpha}$ reducing activity was decreased significantly to about 70% of control levels in response to both HEX and DES, as was 4BP reducing activity. Subsequent administration of hCG to estrogen-treated rats increased both enzyme activities to more than 1.6-fold of the levels of estrogen alone.

Correlation between the activity and content of ovarian CR. Correlation between the reducing activity and the CR content was plotted by using typical substrates for CR, which have been reported up to the present (Fig. 1). 15KD-$PGF_{2\alpha}$ reducing activity was correlated significantly with ovarian CR content ($Y = 6.37X + 142.90$, $r = 0.827$; $P < 0.001$) as was 4BP reducing activity ($Y = 0.35X + 6.80$, $r = 0.813$; $P < 0.01$). None of the menadione, 4-nitroacetophenone and 4-nitrobenzaldehyde reduc-

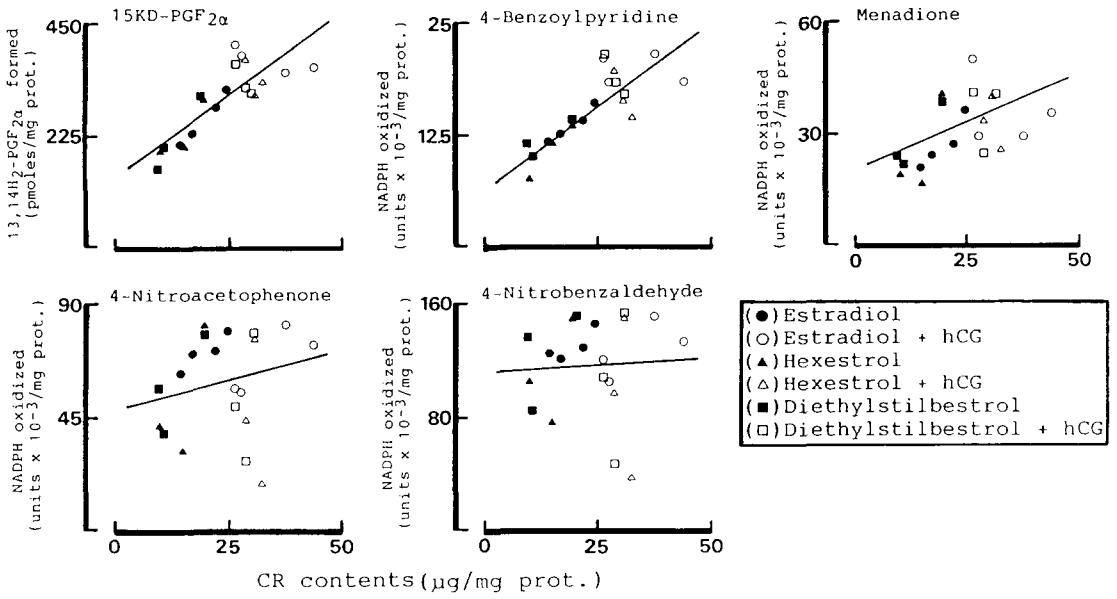

Fig. 1. Correlation between CR activity and CR content. The reductase activity was measured by using 15KD-$PGF_{2\alpha}$, 4BP, menadione, 4-nitroacetophenone and 4-nitrobenzaldehyde as substrates, and the ovarian CR content was determined by western blot-PAP analysis. Each point shows the mean of each group; estradiol: 0 μg/rat (5), 1 μg/rat (8), 10 μg/rat (10), 100 μg/rat (4); estradiol + hCG: 0 μg/rat (5), 1 μg/rat (4), 10 μg/rat (4), 100 μg/rat (4); hexestrol: 0 mg/rat (5), 0.2 mg/rat (8), 2 mg/rat (7); hexestrol + hCG: 0 mg/rat (10), 0.2 mg/rat (6), 2 mg/rat (4); diethylstilbestrol: 0 mg/rat (5), 0.2 mg/rat (9), 2 mg/rat (4); and diethylstilbestrol + hCG: 0 mg/rat (10), 0.2 mg/rat (4), 2 mg/rat (4).

ing activities correlated with the ovarian CR content ($r = 0.457$, $r = 0.192$ and $r = 0.054$, respectively).

Immunohistochemistry. Figure 2 shows the localization of ovarian CR on the day of estrus and after treatment with 0.2 mg HEX. We have already demonstrated the localization of ovarian CR during the estrous cycle [10, 25]. Strong positive immunostaining was observed in both the theca interna cells and the interstitial gland cells on the day of estrus, but the granulosa cells were entirely negative (Fig. 2A). These results are in agreement with our previous reports [10, 25]. Brownish immunoreactivity in the theca interna cells after treatment with HEX was weaker than that on the day of estrus, and immunoreactivity in the interstitial gland cells was faint (Fig. 2B), although various stages of follicles were observed.

DISCUSSION

CR is a member of the aldo-keto reductase family, which includes aldehyde reductase (EC 1.1.1.2) and aldose reductase (EC 1.1.1.21); these are cytosolic, monomeric oxidoreductases that catalyze the NADPH-dependent reduction of a large number of biologically and pharmacologically important endogenous and xenobiotic compounds [1]. We recently purified and characterized CRs from rat ovary [6] and testis [7], which immunochemically cross-react with antibody against human CR [5]. However, immunohistochemical localization of CR in rat ovary and testis with anti-rat ovarian CR antibody is different from that in human ovary and testis with anti-human liver CR antibody [5, 7, 10, 25, 26]. On the other hand, the enzymological properties of rat CRs, which catalyze the reduction of the 9-keto group of PGs and the 3-keto group of steroids, closely resemble those of human CRs [3, 5–7].

We have reported previously that ovarian CR activity is increased markedly after the proestrous LH surge in mature rats, and that ovarian CR is localized in both the theca interna cells and the interstitial gland cells, but not in the granulosa cells [10]. Tamura *et al.* [27] have demonstrated that 17α-hydroxylase/C17-20 lyase is localized in the theca interna cells and the interstitial gland cells, and aromatase in the granulosa cells. It is postulated that these results are consistent with the two-cell theory, namely that androgen produced in the theca interna cells under the regulation of LH is converted to estrogen in the granulosa cells under the control of follicle-stimulating hormone (FSH). Accordingly, our results indicate that ovarian CR is localized in the androgen producing cells and that the regulation is performed by estrogen and LH throughout the estrous cycle.

In immature rats, estrogens including estradiol, HEX and DES increase ovarian CR activity and its content, and potentiate the stimulatory effect of hCG on the activity and content [12, 22, 28]. The purpose of this study was to investigate whether or

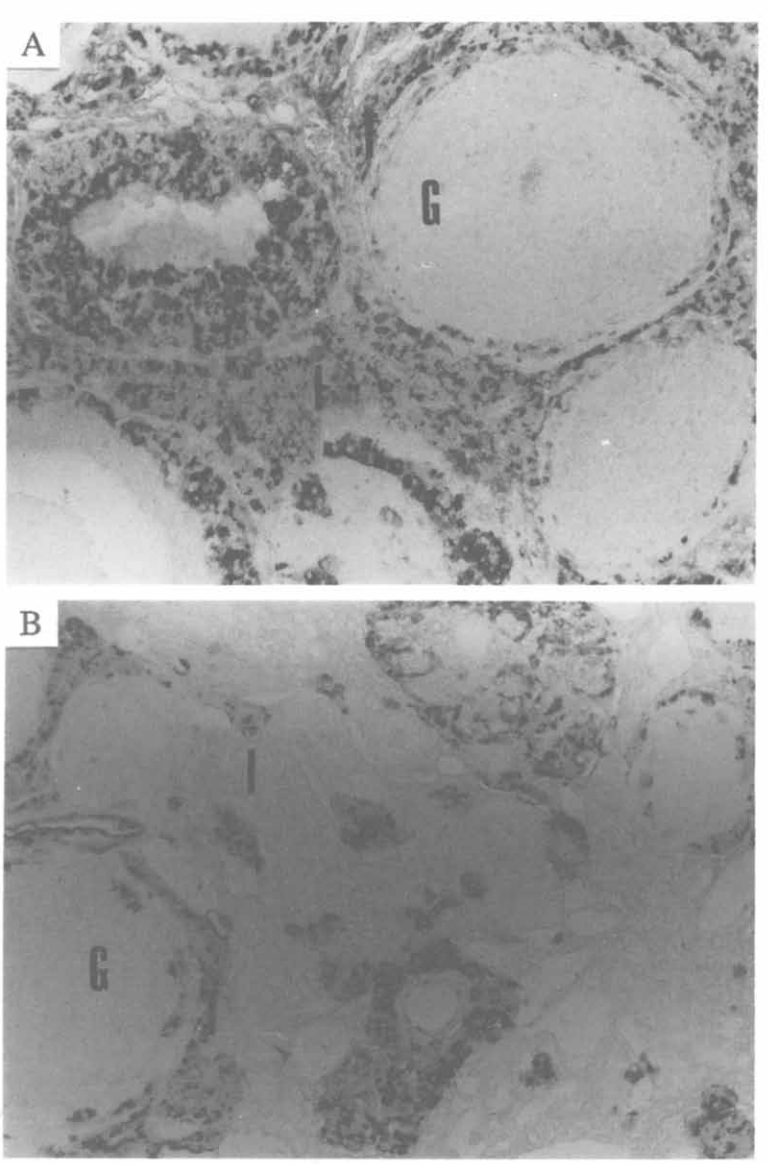

Fig. 2. Immunohistochemical localization of CR in control (estrus) and HEX (0.2 mg/rat)-treated rat ovary. Immunostaining was performed by the ABC method using anti-ovarian CR antiserum. (Panel A) control (estrus); (panel B) HEX-treated. Key: (T) theca interna cells; (G) granulosa cells; and (I) interstitial gland cells. Magnification: ×50.

not ovarian CR activity and its content in mature cycling rats exposed to estrogens are activated by hCG. All of the estrogens tested decreased ovarian weight and inhibited ovulation. In particular, both HEX and DES caused significant decreases in ovarian weight as well as body weight. This appeared to be due to inhibition of gonadotropin release from the pituitary, as previously reported by Strobl and Levine [29], who have concluded that the inhibitory effect of estrogen is exerted by direct action on pituitary gonadotropes. Human CG restored the negative effects of estrogens on ovarian weight and ovulation to the levels of hCG alone, although the number of ova inhibited by estrogens was not restored completely by hCG. These results suggest that estrogen may cause not only inhibition of gonadotropin release from the pituitary but also direct inhibition of the ovulatory process in the ovary.

Changes in the reducing activities towards 15KD-$PGF_{2\alpha}$ and 4BP, which well reflect the ovarian CR activity in rats [6, 10, 11, 25], resembled those in the ovarian CR content. Indeed, both activities correlated well with the ovarian CR content, indicating that quantitative changes in the ovarian CR may affect the enzyme activity. We have also

reported that ovarian CR activity increases after the preovulatory LH surge on the day of proestrus [10]. Accordingly, the significant decrease in the reducing activities of 15KD-$PGF_{2\alpha}$ and 4BP and the ovarian CR content after estrogen treatments may be due to the inhibition of gonadotropin release, including the proestrous LH surge as well as the decrease in the ovarian weight [29]. On the other hand, the significant increase in the enzyme activity and content after hCG treatment in estrogen-treated rats is thought to be due to the expression of the exogenous gonadotropin surge.

It is well established that the PGE and PGF series are closely involved in the ovulatory process and that $PGF_{2\alpha}$ is a factor of luteolysis [30]. We have already demonstrated that $PGF_{2\alpha}$ is metabolized to 13,14H_2-$PGF_{2\alpha}$ via 15-keto-$PGF_{2\alpha}$ and 15KD-$PGF_{2\alpha}$ in rat ovary [31, 32] and that the ovarian CR NADPH-dependently catalyzes the conversion of 15KD-$PGF_{2\alpha}$ to 13,14H_2-$PGF_{2\alpha}$ [6]. It also has been reported that 13,14H_2-$PGF_{2\alpha}$ stimulates the production of estrogen from pregnenolone *in vitro* in the rat ovary [8] and causes luteolysis in heifers [9]. The present results demonstrate that ovarian CR activity and content were decreased by exogenous estrogens and increased by hCG, thus indicating that the formation of 13,14H_2-$PGF_{2\alpha}$ in the rat ovary may be stimulated and inhibited. Furthermore, it is suggested that 13,14H_2-$PGF_{2\alpha}$ is related to the ovulatory process as well as the PGE and PGF series. However, although the ovarian CR activity and content decreased by estrogens was restored by hCG treatment, the number of ova was not restored completely to the levels produced by hCG alone. This result indicates that the increase in the ovarian CR may not always be indispensable to ovulation in mature cycling rats, whereas it is obvious that the enzyme is LH/hCG dependent.

Faint immunoreactivity in the interstitial gland cells of the HEX-treated ovary suggests that exogenous estrogen may predominantly influence the ovarian CR in the interstitial gland cells in mature rats by inhibiting the release of gonadotropin from the pituitary, thus decreasing activity and content.

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