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Expression of the Steroidogenic Acute Regulatory Protein and Luteinizing Hormone Receptor and Their Regulation by Tumor Necrosis Factor α in Rat Corpora Lutea[1]

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ABSTRACT

Expression of both mRNA and protein of the steroidogenic acute regulatory protein (StAR), in correlation with progesterone (P) production and LH receptor (LHR) mRNA expression, was studied in the corpora lutea (CL) of gonadotropin-induced-pseudopregnant and pregnant rats at various stages of CL development. Immature female rats, 21–22 days old, were injected s.c. with 20 IU eCG to stimulate follicle growth and then with 20 IU hCG 48 h later to induce ovulation. The ovaries were removed at various stages of CL development; either CL were isolated and snap frozen for total RNA analysis, or whole ovaries were fixed in Bouin's fluid for paraffin sectioning. The results of in situ hybridization, immunohistochemistry, and Northern blotting showed that the increase in StAR mRNA and protein expression was well correlated with the increase in serum P concentration. StAR expression was restricted to the luteal cells or theca cells in antral follicles. Both StAR mRNA and protein in the CL of pseudopregnant rats increased steadily on Day 1 and Day 4, reached highest levels on Day 4, and then dropped sharply on Day 8 when luteolysis takes place. LHR mRNA content was high on Day 1 but dropped significantly on Day 2. LHR mRNA increased to high levels on Day 4 and 8 and then declined on Day 12. StAR mRNA and protein levels in the CL of pregnant rats were high during early luteal development (Day 2, 4), increased even further on Day 9, and decreased on Day 13 when luteolysis takes place. It is therefore suggested that the expression of StAR coincides well with the capacity of P production in the CL and that StAR expression can be used as a functional "marker" of CL development.

To study the possible effect of cytokines on StAR expression, pseudopregnant rats on Day 5 were injected s.c. with 10 IU hCG plus 20 μg prolactin (PRL), with or without 500 IU tumor necrosis factor α (TNFα) 30 min later. TNFα significantly inhibited hCG/PRL-induced StAR and LHR mRNA expression at 1 and 3 h post-TNFα. It is suggested that the luteolytic effect of TNFα may be mediated by its direct inhibition on StAR expression or by an indirect decrease in LHR expression.

INTRODUCTION

The steroidogenic acute regulatory protein (StAR) is believed to be the key regulator of steroid hormone biosynthesis [1, 2]. De novo synthesis of StAR protein is required for intramitochondrial translocation of cholesterol, the substrate of steroid biosynthesis, to the cytochrome P450 side-chain cleavage enzyme, which is located on the matrix side of the inner mitochondrial membrane [3]. Transfer of cholesterol into the inner mitochondrial membrane is carried out during importation and processing of StAR protein in the mitochondrion [3, 4]. The appearance of StAR has been found to be precisely correlated with steroid production spatially and temporally [5, 6]. The expression of StAR protein in MA-10 mouse Leydig tumor cells and COS-1 cells in the absence of hormone stimulation results in a significant increase in steroid production [3, 7].

StAR has been reported to be present in the ovaries of the mouse [5], rat [8], rabbit [6], human [9], sheep [10], cow [11, 12], and pig [13]. StAR mRNA and protein levels were found to be high in functional corpus luteum (CL) whereas they were absent in regressed CL [12, 14, 15]. Their expression was subject to luteotropic hormones such as eCG, hCG [8], LH [10], and estradiol [6] as well as the luteolytic agent prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) [10, 12, 16].

Tumor necrosis factor α (TNFα) has been demonstrated to be a luteolytic cytokine that is released by leukocytes, macrophages, and endothelial cells within the CL. The secretion level increases during luteal regression in the cow [17] and the sheep [18]. Evidence also shows that TNFα directly inhibits basal and gonadotropin-induced progesterone (P) production in luteal cells [19]. The luteolytic effect of TNFα may be mediated by inhibition of adenylate cyclase [20, 21] and by induction of $PGF_{2\alpha}$ [22, 23]. Because acute regulation of steroid production in the CL is mediated by StAR, and the expression of StAR is subject to regulation by both cAMP [2] and $PGF_{2\alpha}$ [10, 12, 16], TNFα may directly influence the expression of StAR.

Using gonadotropin-induced-pseudopregnant rat and pregnant rat models, we have studied the coexpression of StAR and LH receptor (LHR) in correlation with P production in the CL at various stages. We also investigated the possible effect of TNFα on luteal StAR and LHR expression.

MATERIALS AND METHODS

Animals

Sprague-Dawley rats were obtained from the Institute of Zoology, Chinese Academy of Sciences (Beijing, China) and were fed with chow and water ad libitum. A 14L:10D cycle was maintained with lights-on at 0600 h. One male (about 300 g) and three female (about 250 g) sexually mature rats were put in a cage in the evening. When sperm appeared in the vaginal smear the next morning, that day was considered the first day of pregnancy (Day 1), and the presence of more than one embryo in the uteri was regarded as pregnancy. On Days 2, 4, 9, 13, 18, and 22, the animals were decapitated and ovaries were removed and fixed in Bouin's fluid for in situ hybridization or immunohistochemistry.

Immature female rats (21–22 days old) were injected s.c. with 20 IU eCG (Laboratory Animal Center of Tianjin, Tianjin, China) between 0900 and 1000 h, followed by 20 IU hCG 48 h later. The animals were decapitated 1, 2, 4, 8, and 12 days after injection of hCG (Day 0). Serum was prepared and ovaries were removed; then either the CL

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were isolated and snap frozen for total RNA analysis, or whole ovaries were fixed in Bouin's fluid for in situ hybridization or immunohistochemistry. Some of the pseudopregnant rats were injected s.c. with 10 IU hCG/20 μg prolactin (PRL) on Day 5. Half of these animals were further treated by injection of 500 IU TNFα 30 min later. The animals were decapitated at 1.5, 3.5, and 6.5 h post-hCG/PRL. The ovaries were removed and CL were isolated and snap frozen for total RNA analysis.

RIA

Serum samples were extracted with ether, and the concentration of P was assayed by RIA as previously described [24]. [^{3}H]P was obtained from the Institute of Atomic Power, Chinese Academy of Sciences (Beijing, China). The steroid antisera were prepared as reported previously [25].

In Situ Hybridization

A digoxigenin (DIG) RNA-labeling kit and reagents used for DIG detection, except where noted, were purchased from Boehringer Mannheim GmbH Biochemica (Mannheim, Germany). Mouse StAR cDNA was kindly provided by Dr. Douglas M. Stocco (Texas Tech University Health Science Center, Lubbock, TX). The plasmids were linearized with the appropriate endonucleases and labeled by in vitro transcription. Both antisense and sense StAR and LHR RNA probes were labeled. The ovaries to be used for in situ hybridization were fixed in Bouin's fluid and embedded in paraffin prior to sectioning (4 μm), according to standard procedures. The deparaffinized sections were treated with 8 μg/ml proteinase K (E. Merck, Darmstadt, Germany) for 10 min and washed in PBS for 5 min. Sections were then fixed in 4% paraformaldehyde (Sigma Chemical Co., St. Louis, MO) in PBS for 5 min and washed in PBS for 10 min. Before hybridization, sections were dehydrated through a graded ethanol series and allowed to air dry. The sections were prehybridized with 50% formamide and double-strength SSC (single-strength SSC is 0.15 M sodium chloride and 0.015 M sodium citrate) for 2 h at room temperature; they were then hybridized overnight with DIG-labeled StAR RNA probe in hybridization buffer (10 mM Tris-HCl, pH 7.5, double-strength SSC, 50% deionized formamide, single-strength Denhardt's, 2.5 mM dithiothreitol, 5% dextran sulfate, 250 μg/ml yeast tRNA, and 0.5% SDS) at 48°C. After hybridization, the sections were thoroughly washed in double-strength, single-strength, and 0.1-strength SSC, each twice, for 15 min each time, at 40°C. The sections were then rinsed in DIG buffer I (0.1 M maleic acid, 150 mM NaCl, pH 7.5) for 5 min, blocked with 1% blocking reagent in DIG buffer I for 1 h, incubated with alkaline phosphatase-conjugated anti-DIG IgG diluted 1:500 in DIG buffer I containing 1% blocking reagent for 1 h, and washed in DIG buffer I three times for 5 min each. The bound antibody was detected by a standard immunoalkaline phosphatase reaction, using 5-bromo-4-chloro-3-indolyl phosphate and nitro blue tetrazolium as substrate, for 2–6 h. The sections were dehydrated through a graded series of ethanol, cleared in xylene, and then mounted. For control hybridization, the sections were hybridized with StAR sense RNA probe.

Immunohistochemistry

Immunohistochemistry was carried out with a Vectastain ABC (avidin-biotin-peroxidase) kit (Vector Laboratories, Burlingame, CA) as recommended by the manufacturer. Deparaffinized sections were incubated with 10% normal goat serum (NGS) in PBS for 30 min. The primary antibody to mouse StAR protein, raised in rabbits (kindly provided by Dr. Douglas M. Stocco, Texas Tech University Health Science Center, TX), was diluted in PBS containing 10% NGS and incubated with the sections for 1 h (control groups were incubated with 10% NGS in PBS instead of primary antibodies). Then the sections were washed in PBS three times (5 min each), incubated with biotinylated second antibody for 1 h, and washed in PBS three times (5 min each). After incubation with avidin-biotin-peroxidase complex in PBS for 1 h and washing in PBS three times for 5 min each, sections were incubated in diaminobenzidine tetrahydrochloride in 0.05 M Tris-HCl (pH 7.2) with 0.01% H_2O_2 for 2 to 7 min. Sections were dehydrated and mounted as described above for in situ hybridization.

RNA Isolation and Northern Analysis

Total RNA was extracted from the isolated CL from the ovaries by a single-step acid guanidine thiocyanate-phenol-chloroform procedure [26]. Fifteen micrograms of total RNA was electrophoresed on a formaldehyde-denatured 1% agarose gel, vacuum blotted to a piece of Zeta-Probe nylon membrane (Bio-Rad Laboratories, Hercules, CA) at 40 mbar for 4 h, and cross-linked at 100 mJ by a GS Gene Linker UV chamber (Bio-Rad Laboratories). The membrane was prehybridized in 50% deionized formamide, 5-strength SSC, 0.1% *N*-lauroylsarcosine, 0.02% SDS, 2% blocking reagent (Boehringer Mannheim GmbH Biochemica), and 2% dextran sulfate at 68°C for 2 h; it was then hybridized overnight to DIG-labeled RNA probe (about 300 ng probe in 10 ml prehybridization buffer). After hybridization, the membrane was washed with double-strength SSC two times, 5 min each, at room temperature and 0.1-strength SSC two times, 15 min each, at 68°C. Membranes were then rinsed in DIG buffer I for 1 min and blocked with 1% blocking reagent in DIG buffer I for 30 min, incubated with alkaline phosphatase-conjugated anti-DIG IgG (Boehringer Mannheim GmbH Biochemica) diluted 1:5000 in DIG buffer I containing 1% blocking reagent for 30 min, and washed in DIG buffer I three times for 10 min each. The membrane was then rinsed for 5 min in 0.1 M Tris-HCl, 0.15 M NaCl (pH 9.5) and incubated with CDP-Star chemiluminescence reagent (DuPont, Boston, MA); it was next exposed with Fuji medical x-ray film (Fuji Photo Film Co., Tokyo, Japan) for 2–10 min. The relative contents of StAR or LHR mRNA were obtained by densitometric analysis using a Personal Densitometer SI (Molecular Dynamics, Sunnyvale, CA), corrected for the amount of glyceraldehyde phosphate dehydrogenase (GAPDH), a housekeeping enzyme, or 28S rRNA and averaged for each replicate.

Statistical Analysis

Each experiment was repeated at least twice. Total RNA extraction for Northern blot analysis contained duplicate or triplicate samples. Untransformed data were analyzed by ANOVA. Differences among groups were detected by Tukey's multiple comparison test [27], with differences considered significant if $p < 0.05$.

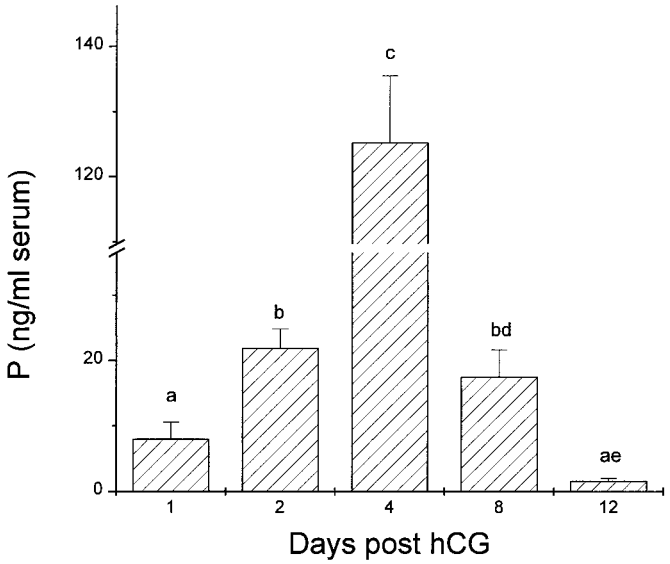


FIG. 1. Serum P levels in the eCG/hCG-induced-pseudopregnant rat. Values are the means ± SEM of more than three separate experiments, analyzed by ANOVA followed by Tukey's multiple comparison test. A different letter denotes a significant difference ($p < 0.05$).

RESULTS

Pseudopregnant Rat Serum P Level

As shown in Figure 1, serum P level was low on Day 1 of pseudopregnancy, increased during the next several days, and reached the maximum level on Day 4. It then dropped significantly on Day 8 and thereafter.

Localization of StAR mRNA and Antigen in Pseudopregnant Rat CL

Both the mRNA (Fig. 2) and antigen (Fig. 3) were restricted to the luteal cells in CL or theca cells in antral follicles at 24 h post-hCG. StAR expression increased significantly in the luteal cells on Days 1 and 4, with the most prominent expression observed on Day 4. On Day 8, both the mRNA and antigen could be barely detected in the CL. StAR mRNA expression, however, was detectable thereafter, but the antigen level still remained low.

Northern Blot of StAR mRNA in Pseudopregnant Rat CL

We observed three rat StAR transcripts, of 1.3 kilobases (kb), 1.6 kb, and 3.5 kb (Fig. 4, upper panel). StAR mRNA level increased steadily in the CL on Day 1 and Day 4, reached the highest level on Day 4, and dropped to the lowest level on Day 8 (Fig. 4). StAR mRNA expression then increased slightly on Day 12. The results obtained by Northern analysis were consistent with the observations made with in situ hybridization and immunohistochemistry.

Northern Blot of LHR mRNA in Pseudopregnant Rat CL

In response to the injection of hCG, LHR mRNA content in CL obtained from pseudopregnant rat ovaries decreased about 40% on Day 2 as compared with values for Day 1 (Fig. 5). The level of the mRNA in the CL then increased dramatically on Day 4, was maintained at this level until Day 8, and dropped on Day 12.

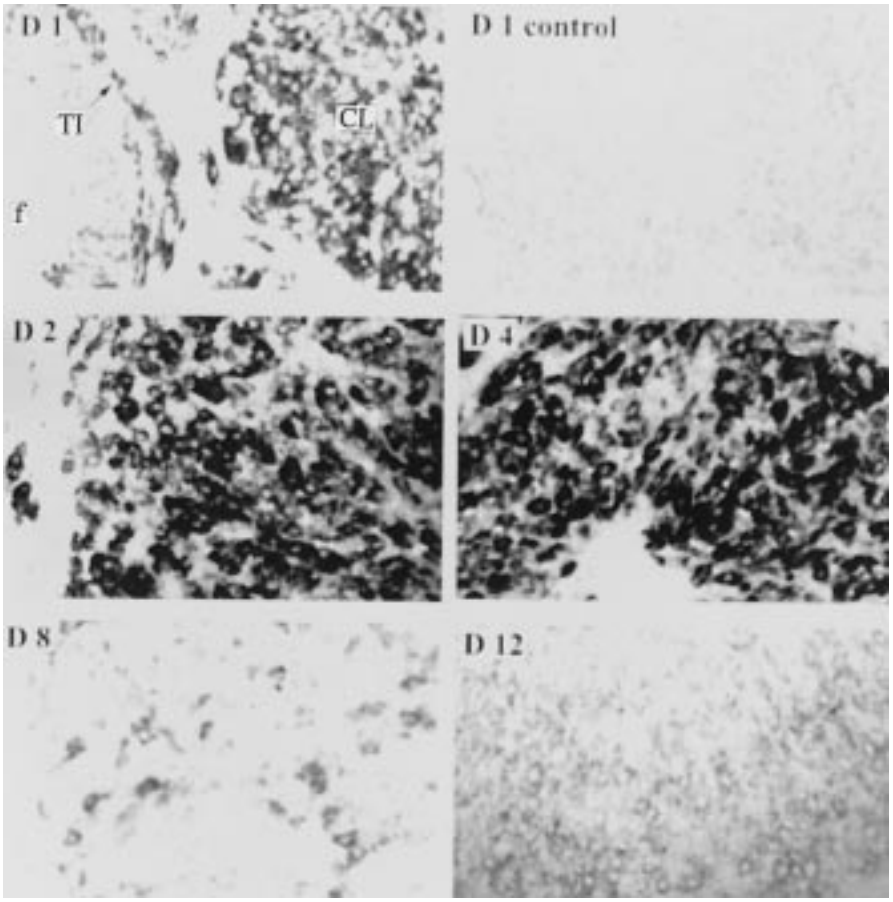


FIG. 2. In situ hybridization of StAR mRNA in the CL of eCG/hCG-induced-pseudopregnant rats. f, Follicle; TI, theca-interstitial cells. All sections were photographed at ×200.

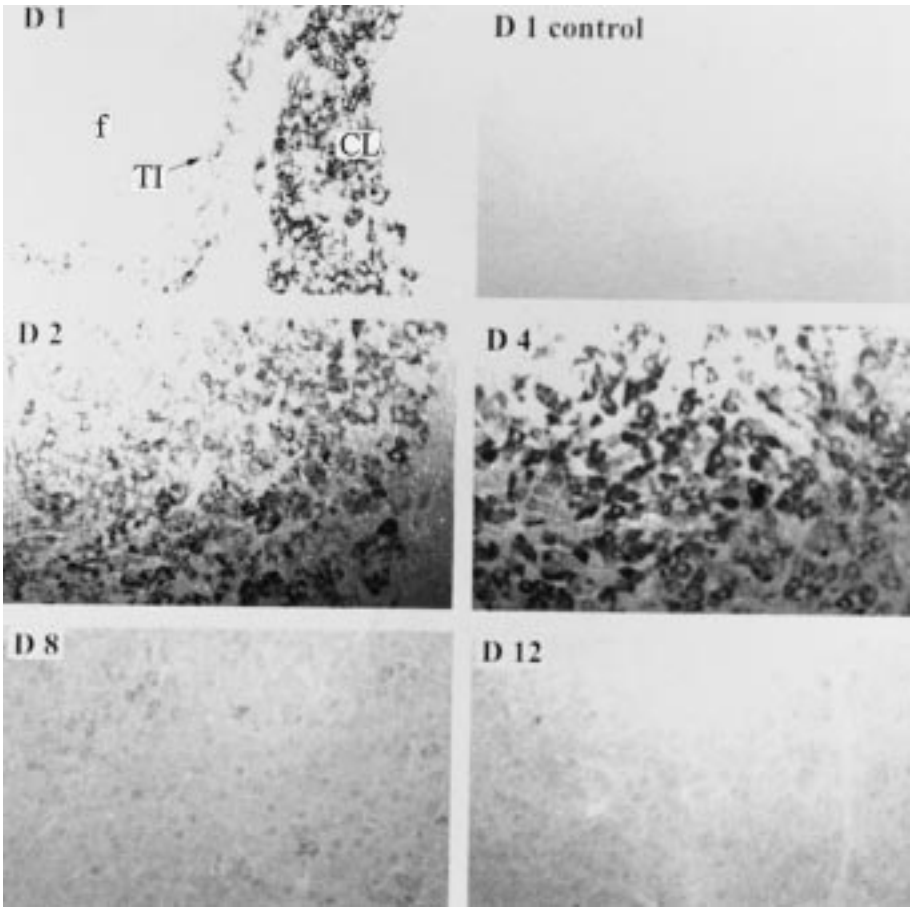


FIG. 3. Immunohistochemical localization of StAR antigen in the CL of eCG/hCG-induced-pseudopregnant rats. f, Follicle; TI, theca-interstitial cells. All sections were photographed at ×200.

Localization of StAR mRNA and Antigen in Pregnant Rat CL

To investigate further whether the expression of StAR mRNA and antigen was correlated with changes in P production in the CL, and to confirm whether expression followed the same profile in the CL induced by injection of exogenous hCG and induced by endogenous LH, we carried out experiments using the pregnant rat model, as shown in Figures 6 and 7. Expression of StAR mRNA and antigen was high on Day 2 and Day 4. These parameters increased further in the CL on Day 9 and then decreased on Day 13 and thereafter. These changes in StAR expression were well correlated with changes in P production in the CL [28].

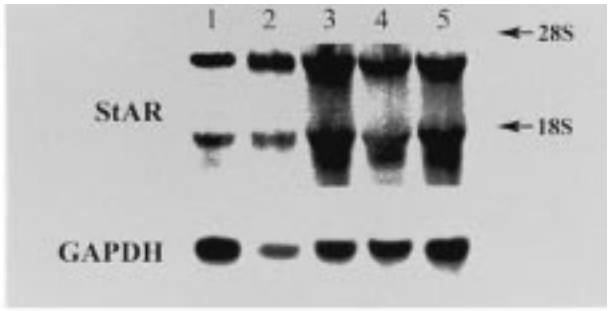
Effect of TNFα on hCG/PRL-Induced StAR and LHR Expression in Pseudopregnant Rat CL

To study whether cytokines can affect hCG/PRL-induced StAR expression in the CL, 10 IU hCG plus 20 μg PRL was given to pseudopregnant rats on Day 5 with or without 500 IU TNFα injection 30 min later. The ovaries were removed at various times, and the CL were isolated for total RNA extraction. As shown in Figure 8, StAR mRNA content in the CL of pseudopregnant rats doubled at 1.5 h after hCG/PRL stimulation and increased further to 2.6 times at 3.5 h, but decreased to the base level at 6.5 h post-hCG/PRL. Injection of TNFα significantly inhibited hCG/PRL-induced StAR mRNA production at 1.5 h and 3.5 h. Furthermore, in the present studies we also observed that injection of hCG/PRL dramatically induced LHR mRNA expression at 1.5 h post-hCG/PRL. Administration of TNFα completely inhibited the increase in LHR mRNA (Fig. 9).

DISCUSSION

We observed three rat StAR transcripts of 1.3, 1.6, and 3.5 kb, consistent with those described previously [8, 29]. In addition to observations in the rat, multiple transcripts for StAR mRNA have been reported for most species studied to date [5, 7, 10, 12]. The difference in transcript length is attributed to variations in the 3′-untranslated regions of the StAR mRNA [11]. Kim et al. [30] and Ariyoshi et al. [31] have reported that alternative polyadenylation sites can account for two mRNAs. There might be translational regulation for StAR expression, and a particular mRNA of StAR multiple transcripts might be preferentially translated [2]. In the present study, we found that StAR protein content was correlated with StAR mRNA in the rat CL, as is the case in the bovine CL [12]. It is therefore suggested that transcription and translation of StAR may be coupled in the CL.

We have demonstrated that the expression of StAR is well correlated with CL steroidogenesis. This is consistent with previous reports [12, 14, 15]. Luteotropic hormones, such as eCG, hCG [8], LH [10], and estradiol [6] can stimulate while $PGF_{2\alpha}$ inhibits StAR expression [10, 12, 16]. Liu and Stocco [32] have recently reported that an increase in heat shock protein-70, which has been shown to appear when luteolysis takes place, is coupled with the decline of StAR expression. It is therefore possible that StAR expression can be used as a functional marker of steroidogenesis. The sharp declines of StAR expression in CL, in correlation



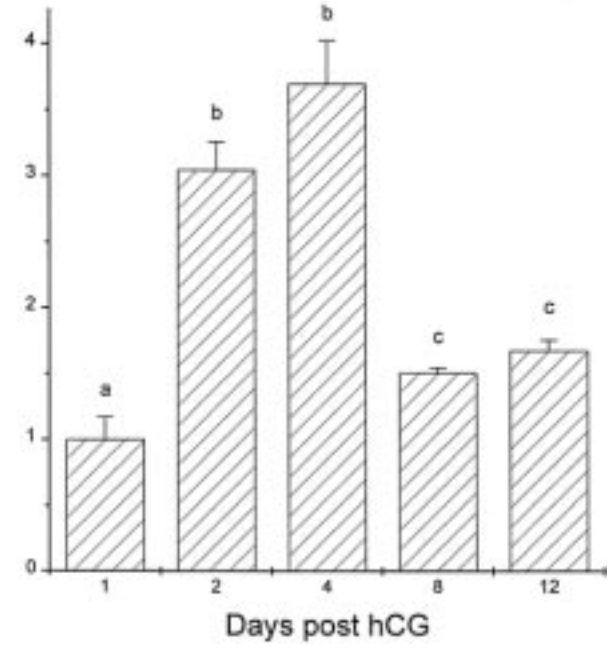
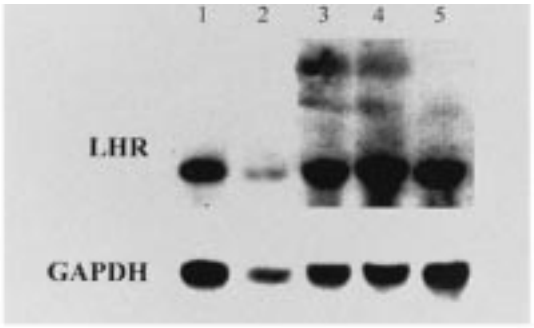


FIG. 4. Expression of StAR mRNA in the CL of pseudopregnant rats. Upper panel: Northern blot hybridization analysis using DIG-labeled StAR RNA probe. Lane 1: Day 1; lane 2: Day 2; lane 3: Day 4; lane 4: Day 8; lane 5: Day 12. Shown below is the same blot reprobed for GAPDH, which was used as a control. Lower panel: Relative contents of StAR mRNA, obtained by densitometric analysis, corrected for the amount of GAPDH and averaged for each replicate. Values are the means ± SEM of three separate experiments. Data were analyzed by ANOVA followed by Tukey's multiple comparison test. A different letter denotes a significant difference ($p < 0.05$).



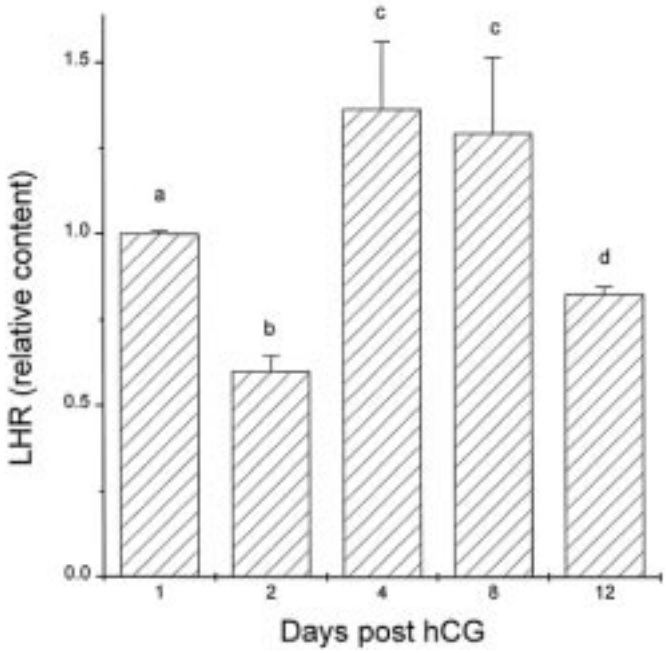


FIG. 5. Expression of LHR mRNA in the CL of pseudopregnant rats. Upper panel: Northern blot hybridization analysis using DIG-labeled LHR RNA probe. Lane 1: Day 1; lane 2: Day 2; lane 3: Day 4; lane 4: Day 8; lane 5: Day 12. Shown below is the same blot reprobed for GAPDH, which was used as a control. Lower panel: Relative contents of LHR mRNA, obtained and corrected for the amount of GAPDH as described for Figure 4. Values are the means ± SEM of three separate experiments. Data were analyzed by ANOVA followed by Tukey's multiple comparison test. A different letter denotes a significant difference ($p < 0.05$).

with a sharp decrease in P production, may be considered the initiation of functional luteolysis.

TNFα has been proven to be one of the luteolytic cytokines [33]. TNFα levels increase during luteal regression [17, 18], perhaps due to the entry of TNFα-producing cells (leukocytes and macrophages) into CL [18]. TNFα could inhibit the luteotropic action of LH/hCG on steroidogenesis and induce CL regression by stimulating release of $PGF_{2\alpha}$ and modulating the protein kinase A signal transduction pathway at multiple levels, including reducing the number of LHR and decreasing adenylate cyclase and protein kinase A activities, cAMP synthesis, and P450 side-chain cleavage mRNA expression [33]. Our study showed an inhibitory effect of TNFα on hCG/PRL-induced StAR and LHR mRNA expression in the CL, indicating that TNFα might play a key role in CL regression. This finding of TNFα inhibition of StAR expression is consistent with a recent report by Mauduit et al. [34]. Those authors found that TNFα inhibits hCG/LH-induced testosterone and StAR expression in cultured porcine Leydig cells, but they did not find any effect of TNFα on StAR and testosterone production before 6-h culture. The difference between our observation and that of Mauduit et al. may be attributable to the animal models used, as the authors suggested [34]. The molecular mechanism of TNFα action on StAR expression may be complicated and still is not clear. TNFα is capable of inhibiting LHR mRNA expression. Because hCG/PRL has an acute effect on LHR mRNA production (Fig. 9), it is possible that the observed inhibitory effect of TNFα on StAR expression may be indirect via reduction in LHR expression. We reported previously that a cytokine, interferon γ, is capable of directly inhibiting P production in the pregnant rat by increasing CL tissue-type plasminogen activator (tPA) activity, which has been reported to play an essential

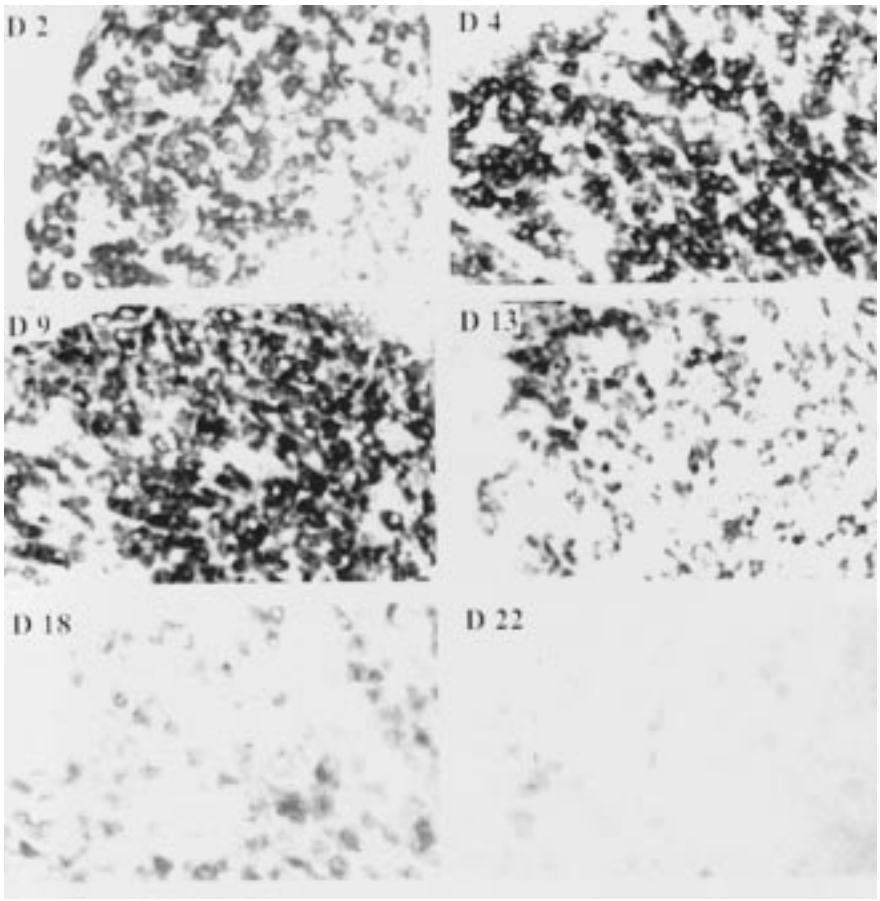


FIG. 6. In situ hybridization of StAR mRNA in the CL of pregnant rats. All sections were photographed at ×200.

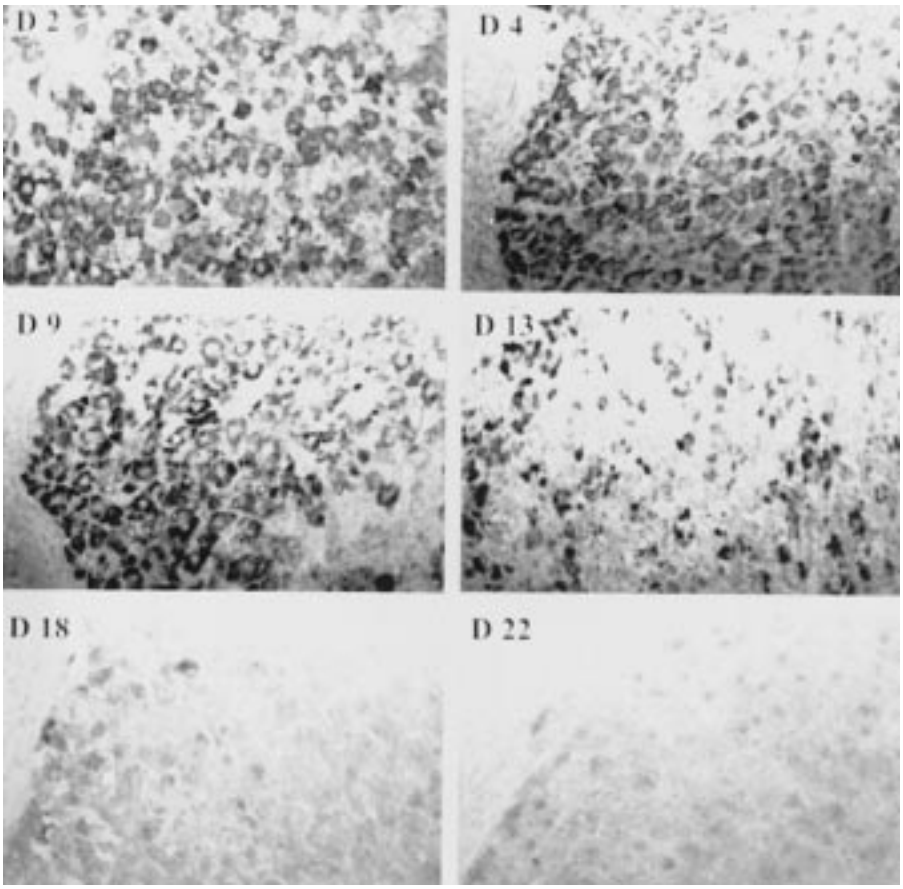
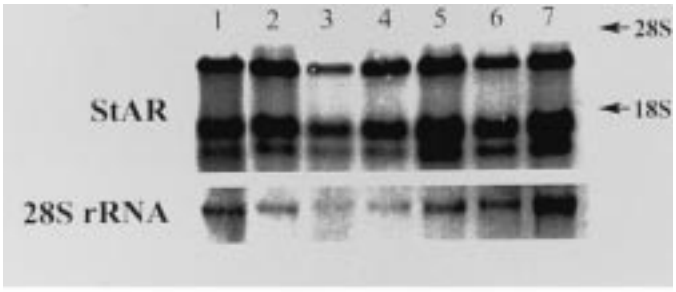


FIG. 7. Immunohistochemical localization of StAR antigen in the CL of pregnant rats. All sections were photographed at ×200.



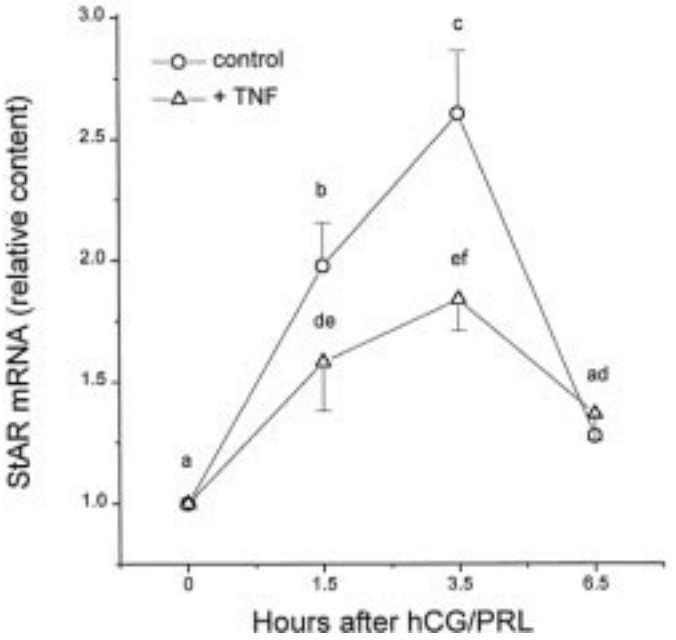
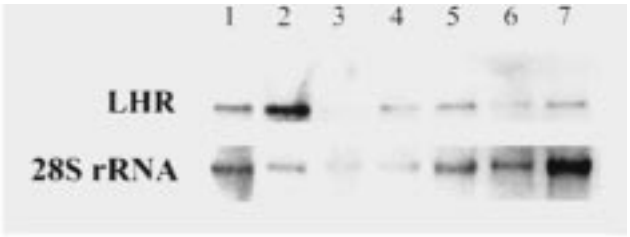


FIG. 8. Effect of TNFα on hCG/PRL-induced StAR mRNA expression in the CL of pseudopregnant rats. Upper panel: Northern blot hybridization analysis using DIG-labeled StAR RNA probe. Lane 1: 0 h; lane 2: 1.5 h, TNFα −; lane 3: 1.5 h, TNFα +; lane 4: 3.5 h, TNFα −; lane 5: 3.5 h, TNFα +; lane 6: 6.5 h, TNFα −; lane 7: 6.5 h, TNFα +. Shown below is 28S rRNA, used as a control. Lower panel: Relative contents of StAR mRNA, which were obtained and corrected for the amount of 28S rRNA. Values are the means ± SEM of three separate experiments. Data were analyzed by ANOVA followed by Tukey's multiple comparison test. Groups with at least one identical letter are not significantly different.



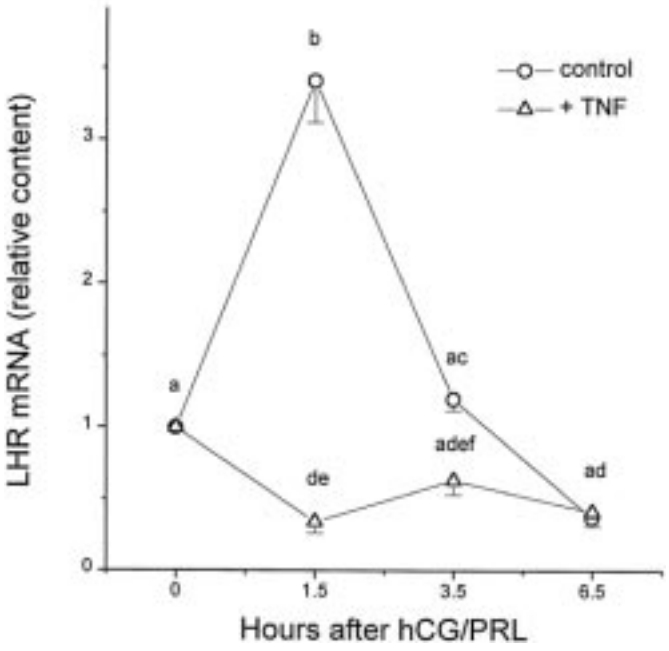


FIG. 9. Effect of TNFα on hCG/PRL-induced LHR mRNA expression in the CL of pseudopregnant rats. Upper panel: Northern blot hybridization analysis using DIG-labeled LHR RNA probe. Lane 1: 0 h; lane 2: 1.5 h, TNFα −; lane 3: 1.5 h, TNFα +; lane 4: 3.5 h, TNFα −; lane 5: 3.5 h, TNFα +; lane 6: 6.5 h, TNFα −; lane 7: 6.5 h, TNFα +. Shown below is 28S rRNA, used as a control. Lower panel: Relative contents of LHR mRNA, which were obtained and corrected for the amount of 28S rRNA. Values are the means ± SEM of three separate experiments. Data were analyzed by ANOVA followed by Tukey's multiple comparison test. Groups with at least one identical letter are not significantly different.

role in modulating extracellular matrix degradation and signal transduction [28]. TNFα has also been proven to stimulate release of the luteolytic factor $PGF_{2\alpha}$, which inhibits P production by activating the protein kinase C signal transduction pathway [35] and enhancing the intracellular level of free calcium [36]. $PGF_{2\alpha}$ and cytokines such as TNFα and interferon γ [22] have long been known as luteolytic factors that could suppress steroidogenesis in CL. Our result suggests that the luteolytic effects of cytokines may be mediated by direct or indirect inhibition of StAR expression.

A variety of factors could influence the number of LHR in CL. Injection of pseudopregnant rats with hCG plus PRL significantly enhances LHR mRNA production. The mechanism of the combined action of hCG with PRL on LHR mRNA induction is not clear. Without PRL, exposure of luteal tissue to high concentrations of LH/hCG invariably results in a dramatic loss of LHR [37, 38]. Our study shows that 48 h after hCG injection, LHR mRNA content in CL dropped significantly. Therefore, LH/hCG could affect LHR number by inhibiting LHR mRNA transcription.

It is thought that once the CL begins to develop, its secretion of P is highly correlated with the number of LHR in the rat [39]. In the CL of the pregnant rat, LHR mRNA content is well correlated with P production and StAR expression, whereas in the pseudopregnant rat this is not the case: though StAR expression had dropped markedly on Day 8 and the serum P concentration began to decline, LHR mRNA levels were still maintained (Fig. 5). While it is clear that LH plays an essential role in CL function, it may not be the only tropic hormone for maintaining CL function. PRL may be an additional important luteotropic hormone in rodents. PRL could increase LHR number in CL, enhance CL steroidogenesis, and decrease sensitivity of the CL to $PGF_{2\alpha}$ [40]. The stimulus of the pelvic nerve during mating could enhance PRL secretion between 8 and 24 h later in the rat [41]. In pregnant rats, PRL, like another protein from the uterus, luteotropin, and androgen and estrogen from the CL or placenta, has been reported to help

maintain CL function after midgestation [42]. In eCG/hCG-induced-pseudopregnant rats, the functional life span of the CL is shorter than that in the pregnant rat [28], and StAR expression as well as P level drops earlier than LHR mRNA expression. This observation might be attributable to the lack of support of luteotropin from the pseudopregnant rat uterus.

Greenwald [43] and others [44] proposed that medium and large antral follicles, which are present throughout gestation, are ''physiologically immature,'' and lack the features typical of steroidogenically active tissue. We found that in the pregnant or pseudopregnant rat ovary, theca cells in preovulatory follicles are capable of expressing StAR. However, neither theca nor granulosa cells in medium or large antral follicles could express StAR during gestation. Our results further confirm that follicles are incapable of steroidogenesis during gestation. These follicles may not be able to develop into ''physiologically mature'' ones during gestation. The inability of follicles to develop further during gestation may be due to the high level of PRL present at this time. Specific PRL receptors have been found in the ovary of several mammalian species [45]. PRL acts directly on developing human follicles to inhibit ovarian steroidogenesis, follicular maturation, and ovulation [46–49]. PRL may inhibit or delay gonadotropin-induced ovulation [50], and at the same time inhibit tPA expression in granulosa cells [51], indicating that PRL may act on the ovary by interfering with the mechanisms causing rupture of the follicle. PRL treatment in vitro has been shown to cause a decrease in ovarian aromatase activity [52, 53].

In summary, StAR expression coincided well with CL steroidogenesis and could be used as a functional marker of rat CL. TNFα could directly or indirectly inhibit hCG/PRL-induced StAR expression. It is suggested that the luteolytic effect of TNFα may be mediated by its inhibition on StAR expression.

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