Modulation of LH/hCG Receptors and Physical State of Ovarian Membranes in Rat Pseudopregnancy

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Abstract. Previous investigations have demonstrated that increased ovarian function during pseudopregnancy in the rat may be associated with alterations of the physical state of membranes Changes in rigidity of membrane lipids were observed during the formation as well as regression of corpora lutea The effects of cyclooxygenase inhibitors (indomethacin and acetylsalicylic acid (ASA)) and of selected steroids (estradiol, testosterone and dihydrotestosterone) on the functional state of luteinized ovaries were studied The compounds were administered to the animals in silastic capsules on different days after hCG injection ASA and indomethacin administration on days 10 and 11 after hCG injection resulted in an increase in the LH/hCG receptor binding activity and rigidity of ovarian membrane lipids, as determined by fluorescence polarization of 1,6-diphenyl-1,3,5 hexatriene (DPH) probe This effect was apparent within 7 days after indomethacin and ASA treatment Both estradiol and testosterone significantly increased the ovarian LH/hCG binding activity, however estradiol did not affect the membrane lipid rigidity Unlike testosterone, the administration of dihydrotestosterone induced a decrease in membrane lipid rigidity and reduced the accessibility of the LH/hCG receptor Inhibitors of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) synthesis, as the endogenous mediator of luteolysis, were shown to delay the regression of the corpora lutea and to prolong the luteal activity in pseudopregnant rats

Key words: LH/hCG receptor — Membrane rigidity — Luteal regression — Rat pseudopregnancy

Introduction

The model of progesterone secretion throughout the process of luteinization consists of a rising phase, a plateau phase and a regression phase The first two phases

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are controlled by the luteotropic process – promotion of progesterone secretion. The regression phase is characterized by functional and morphological changes leading to luteolysis (Rothchild 1981). The formation of the LH/hCG receptor and progesterone in the corpora lutea is regulated by a number of factors of both intra- and extraovarian origin. The physical state of the membrane can be one of these factors. The lipid environment in which the receptor is embedded can affect the accessibility of the receptor as well as transmission of the signal across the membrane. Previously, we have shown that the marked increase of LH/hCG receptors during the formation of corpora lutea was accompanied with an increased rigidity of membrane lipids (Kolena et al. 1990).

$PGF_{2\alpha}$ has been suggested to be the physiological agent responsible for corpus luteum regression in many species, including the rat. The mechanism of luteolysis may involve reduction of corpora lutea LH/hCG receptors (Behrman et al. 1978), alteration of ovarian blood flow (Nett et al. 1976), or inhibition of LH-stimulated cAMP formation and protein kinase activation (Lahav et al. 1989). In pseudopregnant rats, a correlation between luteal concentration of $PGF_{2\alpha}$ and the fall of luteal function has been observed, demonstrating the role of this mediator in rat luteolysis (Cao and Chan 1993).

Conflicting reports exist in the literature as to the effect of steroid hormones on follicular growth and development. Some investigators who used hypophysectomized animals reported stimulation of ovarian function with androgens (Hillier and Ross 1979), others have found inhibition by (Louvet et al. 1975), or no effect of androgens (Kohut et al. 1985).

Dihydrotestosterone specifically inhibited the FSH induction of LH/hCG receptors in granulosa cells (Jia et al. 1985) possibly leading to follicular atresia. On the other hand, estradiol increased ovarian weight and decreased the rate of atresia (Harman et al. 1975).

Since indomethacin and ASA are commonly used inhibitors of prostaglandin synthesis, the *in vivo* effects of these drugs on prolonged function of corpora lutea life were investigated. The results indicate that prolongation of pseudopregnancy is associated with the maintenance of elevated levels of LH/hCG receptors and of rigidity of membrane lipids in luteinized rat ovaries.

Materials and Methods

Materials

Purified hCG (CR 123, 12,780 IU mg^{-1}) was generously supplied by NIAMDD, NIH, Bethesda, USA. $Na^{125}I$ was purchased from the Radiochemical Center, Amersham, UK. Pregnant mare's serum gonadotropin (PMSG), hCG (Praedyn), testosterone isobutyrate (Agovirin-Depot), 17β- estradiol benzoate (Agofollin-Depot), diethylstilbestrol (Difostilben) were from Spofa, Prague, Czech Republic and all other chemicals were from Sigma, Germany.

Methods

Preparation of membranes. Luteinized ovaries were produced in 25-day-old female rats (Wistar strain) by s. c. administration of 50 IU PMSG followed by 30 IU hCG 56 h later (Kolena et al. 1990). Homogenates of ovaries (100 mg ml^{-1}) in ice-cold 50 $mmol.l^{-1}$ Tris-HCl (pH 7.4) were filtered through six layers of surgical gauze, centrifuged at 1 000 × *g* for 15 min, and the supernatant was further centrifuged at 20,000 × *g* for 30 min. The final membrane preparations were resuspended in the same buffer (Kolena et al. 1986).

Preparation of capsules. Silastic tubing (Dow Corning) was filled with a slurry of 2 mg of compounds in 0.15 $mol.l^{-1}$ NaCl, cut in lengths of 15 mm and sealed with silicone plugs. All implants were washed thoroughly before implantation. The capsules were inserted subcutaneously to rats at various times after hCG administration and were left in place as indicated in the legends to Figures (Louvet and Vaitukaitis 1976).

hCG binding assay. In hCG binding assay, 0.1 ml aliquots of ovarian membranes were incubated for 16 h at 20 °C with 0.1 ml PBS (50 $mmol.l^{-1}$ phosphate buffer and 15 $mmol.l^{-1}$ sodium chloride, pH 7.4) + 1 mg ml^{-1} BSA with or without 100-fold excess of unlabeled hCG and 0.1 ml [^{125}I]hCG (1–1.5 ng, spec. act about 2.3 TBq g^{-1}). After the incubation and centrifugation, the membrane pellets were washed twice with PBS buffer (Kolena et al. 1986). The results are expressed as [^{125}I]hCG specific binding per mg ovaries or protein.

Cholesterol, phospholipids and protein assay. Cholesterol was assayed enzymatically (Saté et al. 1984). Phospholipids were determined colorimetrically (as dipalmitoyl phosphatidylcholine) in a complex with ammonium ferrothiocyanate (Stewart 1980). Protein was determined by the method of Lowry et al. (1951).

Fluorescence polarization. Fluorescence polarization was measured with a Perkin-Elmer LS-5 luminescence spectrometer at 25 °C. Solution of 2 $mmol.l^{-1}$ 1,6-diphenyl-1,3,5-hexatriene (DPH) in tetrahydrofuran was dispersed by 1,000-fold agitative dilution in 50 $mmol.l^{-1}$ Tris-HCl buffer, pH 7.4. Ovarian membranes (100 μg protein) were incubated at 25 °C for 1 h with 2 ml of DPH in the above buffer. The fluorescence polarization was computed by equation:

$$P = \frac{I_{vv} - I_{vh}(I_{hv}/I_{hh})}{I_{vv} + I_{vh}(I_{hv}/I_{hh})}$$

where I_{vv} and I_{vh} are fluorescence intensities detected through a polarizer oriented parallelly and perpendicularly to the direction of vertical polarized light, respectively. The I_{hv}/I_{hh} stands for the ratio when the excitation is polarized horizontally and the emission observed through the analyzer oriented perpendicularly and parallelly, respectively (Kolena et al. 1986).

Statistical evaluation

Data were analyzed by ANOVA and Bonferroni's post test Values were considered statistically significant at $P < 0\ 05$

Results

Our previous findings indicated that increased ovarian function in hormone-induced pseudopregnancy in rats may be, at least to a certain extent, associated with an alteration of the physical state of membranes A positive correlation was found between the degree of fluorescence polarization and the accessibility of LH/hCG receptors in ovarian membranes during the formation of corpora lutea (Kolena et al 1990) As the luteolysis proceeded, the days 8–9 after hCG ovulatory injection, the rigidity of ovarian membrane lipids, as determined by fluorescence polarization of DPH probe, decreased (Fig 1*A*, $P < 0\ 01$) The changes in membrane lipid rigidity were similar to the decrease of the content of total and esterified membrane cholesterol as well as of the cholesterol to phospholipids molar ratio (Fig 1*B* $P < 0\ 01$)

The effects of the cyclooxygenase inhibitors (indomethacin and ASA) and selected steroids (estradiol, testosterone and dihydrotestosterone) on the functional state of luteinized ovaries were studied The compounds were administered to the rats in silastic capsules The silastic capsules provide a means of chronic administration of constant amounts of various compounds for long periods of time with

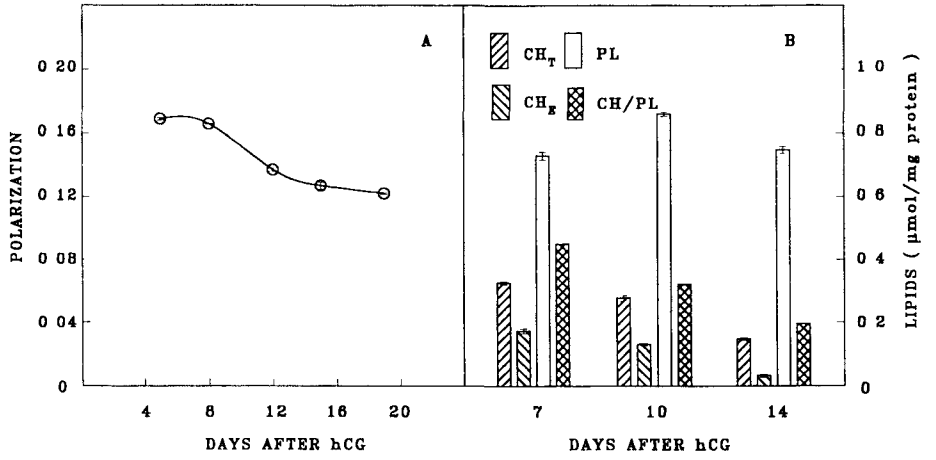

Figure 1. Changes in fluorescence polarization of DPH probe (4) and in cholesterol (total CH_T and esterified CH_E) to phospholipids molar ratios (*B*) in ovarian membranes of rats treated with PMSG and hCG Each value represents the mean ± S E of 4 estimations with 4 6 animals at each time point in each experiment The results were confirmed in 2 independent experiments

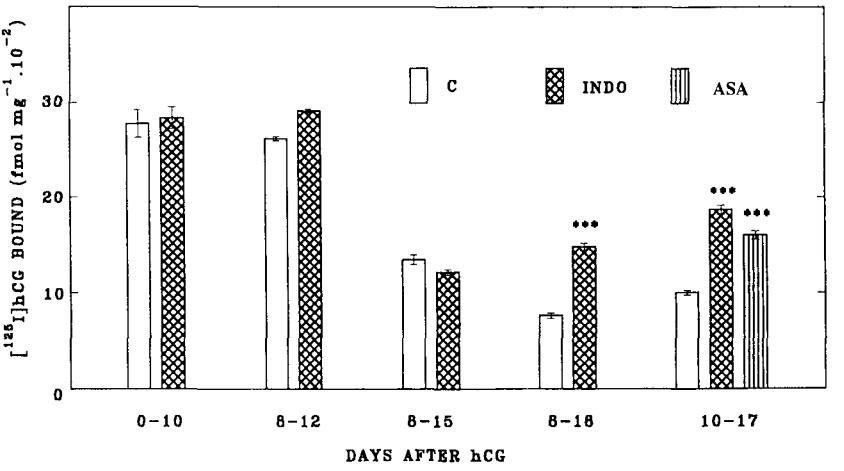

Figure 2. Effects of indomethacin (INDO) and acetylsalicylic acid (ASA) on the accessibility of the rat ovarian LH/hCG receptor during PMSG and hCG induced pseudopregnancy Cyclooxygenase inhibitors were administered subcutaneously in silastic capsules (2mg of compounds in 0 15 mol l^{-1} NaCl) for the indicated periods on different days after hCG injection Each value represents the mean ± S E of 4 estimations in 3–7 rats The results were confirmed in 2 independent experiments

definite onset and cutoff points The rates of diffusion of ^{14}C-labelled 17β-estradiol or testosterone from capsules into buffer or the release of unlabelled steroids into rat plasma appeared to be constant in our experiments (data not shown). Parallel changes in the binding activity of the LH/hCG receptor and rigidity of ovarian membrane lipids were observed after the treatment of rats with indomethacin and ASA during pseudopregnancy. As shown in Fig. 2, specific binding of [^{125}I]hCG to ovarian membranes decreased through the second and third week after hCG injection. At that time, on days 17 and 18, indomethacin and ASA significantly increased the accessibility of ovarian LH/hCG receptors (Fig. 2). The degree of fluorescence polarization of the DPH probe was increased by the action of ASA and indomethacin on day 17 and 18 respectively, after hCG administration (Fig. 4). The results indicate that changes in the physical state of ovarian membranes may be involved in the action of cyclooxygenase inhibitors on ovarian function at the end of hormone-induced pseudopregnancy in rat.

Administration of estradiol to rats during regression of the corpora lutea significantly increased the accessibility of LH/hCG receptors on days 14 and 18 after hCG injection (Fig. 3), but failed to change membrane lipid rigidity (Fig. 4). Diethylstilbestrol, a non-steroidal synthetic estrogen, increased the binding activity of the receptor until day 18 (Fig. 3).

Administration of testosterone or dihydrotestosterone for one week decreased the degree of fluorescence polarization of the DPH probe on day 12 after hCG

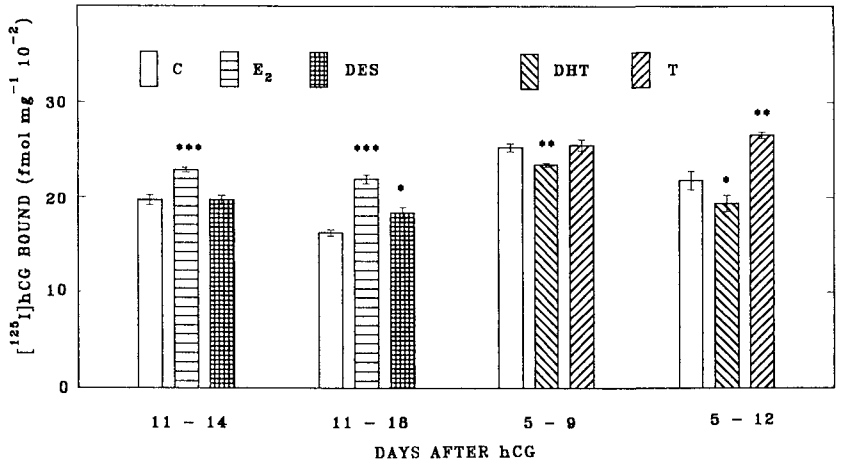

Figure 3. Specific binding of [^{125}I]hCG to ovarian membrane preparations of pseudopregnant rats treated with estradiol (E), diethylstilbestrol (DES), dihydrotestosterone (DHT) or testosterone (T) administered subcutaneously in silastic capsules on various days after hCG Values are means ± S E of 4 estimations (each repeated twice) with 4–6 animals per experiment

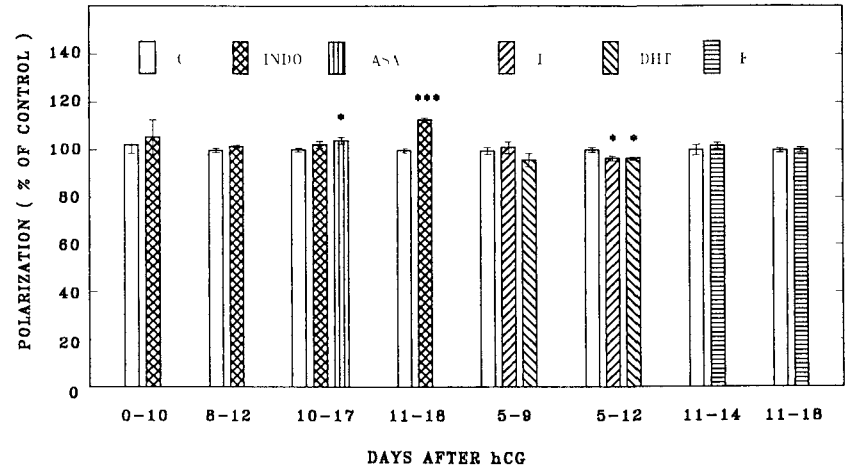

Figure 4. Changes in fluorescence polarization of DPH probe in ovarian membranes of pseudopregnant rats after indomethacin (INDO), acetylsalicylic acid (ASA), testosterone (T), dihydrotestosterone (DHT) or estradiol (E) treatment The respective compounds were administered subcutaneously in silastic capsules on different days after hCG injection Data are means ± S E of 4 determinations ($n = 4$ 6 animals/treatment) The results were confirmed in 2 independent experiments

(Fig 4), while they did not display any effect during shorter periods (4 days) The individual effects of testosterone and dihydrotestosterone on the accessibility of the LH/hCG receptor were different and varied according to the duration of the hormone action Testosterone administration for 7 days increased the specific binding of [^{125}I]hCG to ovarian membranes until day 12 after hCG, while dihydrotestosterone decreased the binding activity of the receptor on day 9 and 12 after hCG injection (Fig 3)

Discussion

The effects of gonadotropins on follicular maturation and luteinization are associated with dynamic changes in ovarian LH receptor content At the time of luteolysis, the decrease in serum progesterone precedes the decline in LH receptors in luteal tissue A similar relationship between corpus luteum LH/hCG receptors and progesterone secretion appears to exist throughout pregnancy in the rat (Kolena et al 1977)

In our previous studies (Kolena et al 1990) as well as in the present study, correlation was observed between LH/hCG receptors and plasma progesterone concentrations ($r = 0\ 98$) and membrane lipid rigidity ($r = 0\ 68$) during pseudopregnancy The changes in membrane lipid rigidity during pseudopregnancy are the apparent result of an alteration in the cholesterol to phospholipids ratio Under physiological condition and with naturally occurring phospholipids, any rise in the cholesterol/phospholipids ratio will be associated with an increase in membrane rigidity (Shinitzky and Inbar 1976) It is obvious that the alteration of LH/hCG receptors in luteinized ovaries reflects the formation of receptor molecules, however, increased membrane lipid rigidity may maximally expose receptors maintained in a cryptic form (Kolena and Kasal 1989) The decreased membrane lipid rigidity along with the accessibility of LH/hCG receptors in luteal membranes during luteolysis appear to be in agreement with the concept of vertical displacement of membrane proteins (Borochov and Shinitzky 1976) According to this concept, the bulk of membrane proteins becomes more exposed to the aqueous medium upon increases in membrane rigidity

The luteolytic effect of $PGF_{2\alpha}$ in rats has been studied for a long time, but the exact mechanism of action remains unknown Treatment with exogenous $PGF_{2\alpha}$ induces functional luteolysis which consists of a rapid desensitization and eventual loss of LH receptors within 12–24h (Behrman et al 1978) Inhibition of prostaglandin synthesis by indomethacin prolongs the luteal life span and increases LH receptor mRNA levels in the rat (Bjurulf et al 1994) $PGF_{2\alpha}$-induced luteolysis was shown to be correlated with a change in phase composition and fluidity of luteal cell membranes (Goodsaid-Zalduondo et al 1982) The results of this study show that cyclooxygenase inhibitors significantly altered LH/hCG receptor binding activity and membrane lipid rigidity ASA and indomethacin administration to rats on day 10 and 11 after hCG injection, resulted in an increase in the LH/hCG receptor binding activity and rigidity of ovarian membrane lipids The effect of

indomethacin and ASA treatment was observed only during the luteolytic, but not luteal phase This is in agreement with the findings that young corpora lutea show resistance to the luteolytic action of $PGF_{2\alpha}$ In young corpora lutea of the rat, $PGF_{2\alpha}$ fails to decrease progesterone production, hCG binding (Hichens et al 1974), LH-dependent cAMP production (Khan et al 1979) and the induction of 20α hydroxysteroid dehydrogenase (Lamprecht et al 1975)

One of the main functions of the corpus luteum is the secretion of progesterone The role of estradiol in luteal steroidogenesis has been extensively investigated in the pregnant rat Estradiol increased the supply of cholesterol substrate by mobilizing cholesterol storage, enhancing luteal cell content of lipoprotein receptors and thus uptake of circulating cholesterol (Khan et al 1985), and by stimulating cholesterol synthesis (Azhar et al 1985) Estradiol also enhanced the transport of cholesterol to mitochondrial $P450_{scc}$ (McLean et al 1989) *In vivo* treatment with estradiol or testosterone prevented the drop in progesterone production and maintained the concentration of serum progesterone at levels found in intact pregnant rats (Khan et al 1987) In the present study, both estradiol and testosterone significantly increased the ovarian LH/hCG binding activity, however estradiol did not affect membrane lipid rigidity indicating that the effect of estradiol on the accessibility of ovarian LH/hCG receptor was not due to changes of the physical state of membranes

On the other hand, Sridaran and Gibori (1981) demonstrated that dihydrotestosterone treatment of the pregnant rat had no effect on serum levels or on ovarian vein concentration of estradiol, but induced a significant decrease in the ovarian vein levels of progesterone Dihydrotestosterone levels in the ovaries were increased significantly between days 18 and 22 of pregnancy, concomitant with the cessation of corpus luteum function The role of dihydrotestosterone has also been demonstrated during follicular growth Dihydrotestosterone significantly reduced the ovulation rate and caused an increase in atresia among secondary follicles (Conway et al 1990) The latter authors have concluded that dihydrotestosterone is acting directly on the follicles and not by acting on the pituitary to suppress the gonadotropin surge In the studies presented herein, administration of dihydrotestosterone to PMSG/hCG-primed rats led to a decrease in membrane lipid rigidity and accessibility of the LH/hCG receptor, indicating that dihydrotestosterone could be involved in the luteolytic process in pseudopregnant rats, and this luteolytic activity of dihydrotestosterone is associated with changes of the physical state of ovarian membranes

The results of this study indicate that the cyclooxygenase inhibitors indomethacin and acetylsalicylic acid partially delayed the regression of corpora lutea and prolonged the luteal activity in pseudopregnant rats by preventing the effect of $PGF_{2\alpha}$, as the endogenous mediator of luteolysis

Acknowledgements. This work was supported, in part, by Grant 2/4134/97 from VEGA and WHO Grant 81077

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Final version accepted October 4, 1999