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RAPID COMMUNICATION

Changes in Physical State of Ovarian Membranes During Pseudopregnancy in the Rat

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Summary

The role of the physical state of ovarian membranes was studied in immature rats after PMSG- and hCG-induced pseudopregnancy. Parallel changes in LH/hCG receptors, progesterone secretion and rigidity of membrane lipids were observed during pseudopregnancy. Possible structure-functional properties of the LH/hCG receptor were analyzed by the thermal perturbation technique. Thermal stability of the receptor was higher 5 days after hCG ovulatory injection to rats compared to days 11 or 18 and to control rats. Pseudopregnancy modified the quenching of protein fluorescence. The Stern-Volmer constants for controls and for rats on days 5, 11 and 18 of pseudopregnancy were found to be 2.4 and 4.6, 5.1 and 4.4, respectively, indicating that accessibility of fluorophores for the quencher was increased.

Key words

LH/hCG receptors – Thermal inactivation – Membrane rigidity – Fluorescence quenching – Rat pseudopregnancy

The formation of the LH/hCG receptor and progesterone in the corpora lutea is regulated by a number of stimulatory and inhibitory factors of both intra- and extraovarian origin. The order of membrane lipids 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. The function of serotonin, insulin, prolactin and gonadotropin receptors was found to be influenced by changes in membrane lipid rigidity (Heron *et al.* 1980, Ginsberg *et al.* 1981, Dave and Witorsch 1983, Kolena and Ondriaš 1984, Kolena *et al.* 1994). Hormonal induction of pseudopregnancy in immature rats provides a well characterized model for the study of mechanisms underlying the control of luteal function. Data are presented here to document that the development of pseudopregnancy in rat ovaries is accompanied by changes in the physical characteristics of the membrane.

Luteinized ovaries were produced in 26-day-old rats (Wistar strain) by s.c. administration of 50 IU PMSG followed 56 h later by 30 IU hCG. Homogenates of ovaries in buffer A (25 mmol.l^{-1} NaH_2PO_4, 1 mmol.l^{-1} EDTA, 40 mmol.l^{-1} NaCl, pH 7.4) were centrifuged at 1000 x *g* for 15 min and the supernatant was further centrifuged at 20 000 x *g* for 30 min. The final membrane preparations were resuspended in the same buffer (Kolena *et al.* 1994). In the hCG binding assay, 0.1 ml of ovarian membranes was incubated for 16 h at 23 °C with 0.1 ml buffer A + 1 mg.ml^{-1} BSA with or without a 100-fold excess of unlabelled hCG and 0.1 ml [^{125}I]hCG (1–1.5 ng, specific activity about 2.3 TBq.g^{-1}). After incubation and centrifugation, the membrane pellets were washed twice with buffer A. Aliquots of membrane-bound LH/hCG receptors were heat-inactivated in a water

bath at a constant temperature of 50 °C for different periods of time (Kolena *et al.* 1994). Fluorescence quenching studies are able to provide valuable information concerning the exposure of tryptophan and tzrosine residues and the dynamics of the protein matrix surrounding such residues. Quenching studies were carried out at 23 °C by adding small amounts of 5 M acrylamide in buffer A, pH 7.4. Fluorescence intensity was measured as a function of the quencher concentration at a fixed emission wavelength of 394, 389 and 380 nm. An excitation wavelength of 280 nm was used. The Stern-Volmer quenching constant K_{sv} was calculated according to the equation $F_o/F = 1 + K_{sv}[Q]$, where F_o is the fluorescence of the unquenched fluorophore and F is the fluorescence at quencher concentration [Q] (Efting and Ghiron 1976).

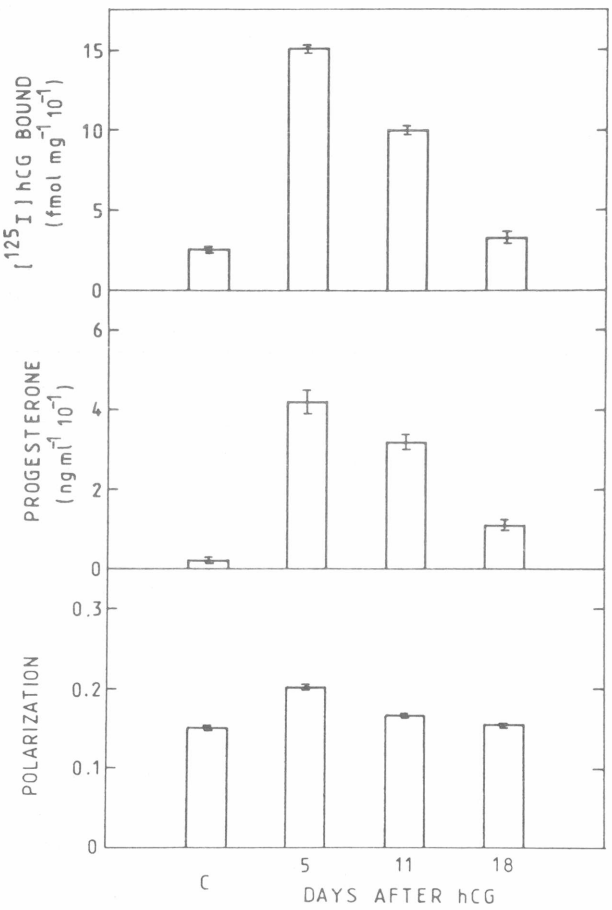

Fig. 1. *Specific binding of [^{125}I]hCG to membrane preparations, plasma level of progesterone and fluorescence polarization of DPH in ovarian membranes during pseudopregnancy in rats. Each value represents data (± S.E.M.) obtained from 4–6 animals. Control rats (C) were 32 days old.*

Fluorescence polarization of DPH was measured on a Perkin-Elmer LS-5 luminescence spectrometer, equipped with a circulation bath to maintain the sample temperature at 2 °C. A solution of 2 mM DPH in tetrahydrofuran was dispersed by 1000-fold agitive dilution in buffer A (pH 7.4). Ovarian membranes (100 μg protein) were incubated at 23 °C for 1 h with 2 ml of DPH in the above buffer (Kolena *et al.* 1994). The RIA method was used for determination of progesterone (Petr *et al.* 1991). The data were analyzed by ANOVA and the Bonferroni post test. The squares method was used to calculate the K_{sv} constant.

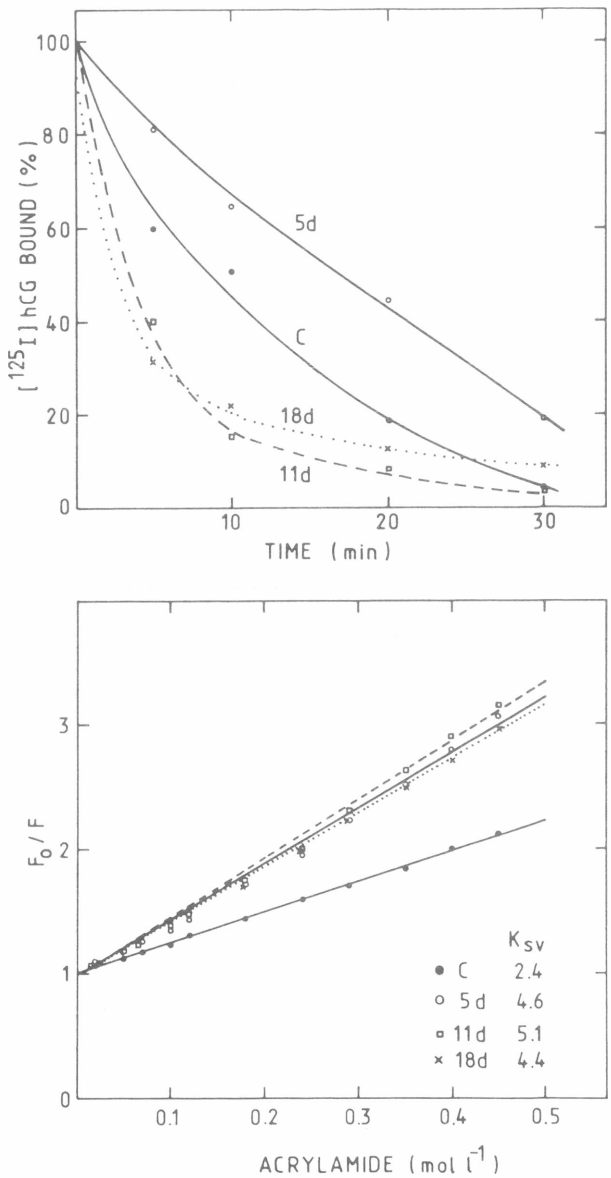

Fig. 2. *Time-dependent thermal inactivation of LH/hCG binding sites at a constant temperature of 50 °C(upper panel) and Stern-Volmer plots with the corresponding Stern-Volmer constants (K_{sv}) of acrylamide quenching (lower panel) of ovarian membranes of control rats or rats 5, 11 and 18 days after hCG treatment. F_o and F are fluorescence intensities measured in the absence and in the presence of acrylamide. The results were confirmed in 2 independent experiments.*

Consistently with previous findings (Lee *et al.* 1975), parallel changes in LH/hCG receptors and progesterone secretion occurred during pseudopregnancy in rats (Fig. 1). In ovaries from PMSG-hCG stimulated rats, luteal cells comprised 70 % of the total ovary, as measured by the DNA content (Kim and Freenwald 1986). Three periods can be distinguished during the existence of corpora lutea: rising phase, plateau and regression (Rothchild 1981). The first two phases are controlled by the luteotropic process, i.e. promotion of progesterone secretion. During the formation of corpora lutea, the affinity of binding sites for hCG was not affected, and the marked increase of LH/hCG receptors was associated with alteration in receptor number (Lee *et al.* 1975). A significant positive correlation ($r=0.92$, $P<0.05$) found between the degree of fluorescence polarization and hCG binding activity indicated that the ordering of membrane lipids could affect the accessibility of the LH/hCG receptor. It is obvious that the increase of LH/hCG receptors in luteinized ovaries reflects the synthesis of new receptor molecules, however, increased rigidity of membrane lipids may maximally expose receptors in a cryptic form (Kolena and Kasal 1989). The increased accessibility of LH/hCG receptor in luteal membranes appears to be in agreement with the concept of vertical displacement of membrane proteins (Borochov and Shinitzky 1976). The bulk of membrane proteins would thus become more exposed to the aqueous medium by increasing membrane rigidity.

Studies of heat inactivation of hCG binding sites were carried out to monitor the structural alteration of the LH/hCG receptor (Villar *et al.* 1988, Kolena *et al.* 1994). Thermal inactivation of the receptor is a rapid process. During incubation of membranes at a constant temperature of 50 °C, the damage of binding sites was appreciably manifested after 5 min, with subsequently increasing severity (Fig. 2). Heat inactivation of the LH/hCG receptor was significantly higher in ovarian membranes on days 11 and 18 of pseudopregnancy than in control membranes. On the other hand, thermal stability of the receptor against thermal perturbation was higher in rats 5 days after hCG ovulatory injection, i.e. at the time when the functional state of the ovary is higher.

Structural and functional changes of the receptor during pseudopregnancy of rats were also reflected by the information obtained in quenching studies. The intrinsic fluorescence of protein appears to be a very valuable probe monitoring protein conformation and protein-lipid interaction. We used acrylamide, a neutral dynamic quencher, to establish whether rat pseudopregnancy modifies the quenching of protein fluorescence. The Stern-Volmer plots are shown in Figure 2. Corresponding Stern-Volmer constants determined from the slopes for control membranes and for those of 5, 11 and 18 days of pseudopregnancy were found to be 2.4 and 4.6, 5.1 and 4.4, respectively, indicating that accessibility of fluorophores for acrylamide was increased. Increased quenching generally suggests an increase in the proximity of quencher molecules to the fluorophore (Efting and Ghiron 1976). It is generally believed that membrane structure as well as molecular order and dynamics are essential for the maintenance of membrane function. Our findings indicate that increased ovarian function in hormone-induced pseudopregnancy of the rat may be, at least to a certain extent, associated with the alteration of the physical state of membranes.

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