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Early hCG-induced desensitization of rat ovarian LH/hCG receptors is associated with altered physical state of membranes

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Abstract

Injection of pseudopregnant rats with pharmacological doses of hCG produced desensitization of adenylylcyclase and steroidogenic systems in ovarian membranes and luteal cells. Membrane lipid rigidity, as determined by fluorescence polarization of DPH, decreased as early as 0.5 h after injection of hCG. Desensitization also modified the differential scanning calorimetric profile characteristic of control membranes. The accessibility of ovarian LH/hCG receptors was unchanged. The results indicate that the hCG-induced decrease of membrane lipid rigidity is preceded by the process of desensitization of the rat ovary.

Key words: LH/hCG receptor; Membrane fluidity; Desensitization; Adenylylcyclase; Rat ovary

1. Introduction

Desensitization is generally defined as the reduction of a biological response to continuous or repetitive stimulation by agonists. This appears to play an important role in modulating cellular signal transduction processes. The mechanisms involved in desensitization have been studied at the level of the receptor-G_s protein stimulated adenylylcyclase system, subdivided into at least two principal categories termed uncoupling and down-regulation. Down-regulation involves a decrease in the number of receptors at the cell surface, a decline in the rate of receptor synthesis and enhanced receptor degradation [1–3]. Uncoupling reflects an agonist-induced change in the functional properties of receptors. Agonists trigger sequestration of receptors away from the plasma membrane. The receptors are still detectable by ligand binding and/or they remain located in membranes, yet they are functionally uncoupled from G_s protein [4–6]. A large body of evidence suggests that this uncoupling, especially in β_2-adrenergic receptors, is caused by phosphorylation of the receptors [7]. But a similar mechanism of phosphorylation, involving the cAMP-dependent protein kinase, appears to be unlikely in the uncoupling of the LH/hCG receptor [3,4]. The cell membrane is a dynamic matrix which responds to various physiological conditions by changing its physical state. The ordering of the lipid environment in which the LH/hCG receptor is embedded can affect the accessibility of the receptor, as well as transmission of the signal across the membrane [8,9]. There are several reports showing that in vitro additions of insulin and growth hormone can elicit changes in the membrane lipid fluidity in adipocytes and erythrocytes [10–12]. The present study provides evidence that the process of short-term desensitization of luteinized rat ovaries following injection of hCG is accompanied by changes in the order of membrane lipids.

2. Materials and methods

Purified hCG (CR 123; 12 780 U/mg) was generously supplied by NIAMDD, NIH, Bethesda. $Na^{125}I$ was purchased from the Radiochemical Centre, Amersham. Creatine phosphate, creatine kinase and GTP-γ-S were obtained from Boehringer-Mannheim. All other chemicals were from Sigma.

Luteinized ovaries were produced in 26-day-old rats (Wistar strain) by sc administration of 50 IU PMSG followed 56 h later by 30 IU hCG [9]. Desensitization was induced by injecting 75 IU hCG on day 5 of pseudopregnancy; control rats received saline. 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) containing 20% sucrose were centrifuged at 1,000 × g for 15 min and the supernatant was further centrifuged at 20,000 × g for 30 min. Plasma membranes were prepared on the sucrose gradient [8,13]. Reconstitution of the ovarian LH/hCG receptor into proteoliposomes after removal of sodium cholate detergent by absorption on Bio-Beads SM-2 was described previously [13,14]. Isolated luteal cells were prepared by enzymatic dispersion of luteinized ovaries with collagenase [15].

In the hCG binding assay, 0.1 ml of ovarian membranes or proteoliposomes were incubated for 16 h at 20°C with 0.1 ml buffer A + 1 mg · ml^{-1} BSA with or without a 100-fold excess of unlabeled hCG and 0.1 ml [^{125}I]hCG (1–1.5 ng, sp. act. about 2.3 TBq · g^{-1}).

After incubation and centrifugation, the membrane pellets were washed twice with buffer A. The hormone–receptor complex in proteoliposomes was precipitated twice with polyethylene glycol [8,13].

Fluorescence polarization was measured with Perkin-Elmer LS-5 luminescence spectrometer at 25°C. A solution of 2 mmol · l^{-1} DPH in tetrahydrofuran was dispersed by 1,000-fold agitative dilution in 50 mmol · l^{-1} phosphate 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 [2,8,9]. Differential scanning calorimetry (DSC) measurements were performed on a Privalov DASM-4 adiabatic differential microcalorimeter with a scan 0.5°C/min. The DSC experiments were carried out

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at pH 7.4 with protein concentration of about 1 mg · ml^{-1} in degassed buffer A [16].

Adenylylcyclase activity was assayed at 30°C for 25 min with 30–40 μg plasma membrane protein [14,17]. RIA and protein binding methods were used for the determination of progesterone and cAMP concentration [8,9]. Cholesterol was assayed enzymatically [18]. Phospholipids were determined colorimetrically in a complex with ammonium ferrothiocyanate [19]. Protein was determined by the method of Lowry et al. [20]. Student's *t*-test was used for statistical evaluation.

3. Results and discussion

In an in vivo experimental model, the present study shows that hCG-induced decrease membrane lipid rigidity is preceded by ligand-induced changes in ovarian luteal LH/hCG receptor content and desensitization of adenylylcyclase activity. Administration of 75 IU of hCG to rats on day 5 of pseudopregnancy resulted in a time-dependent decrease of the accessibility of LH/hCG receptors (Fig. 1). A significant decline in [^{125}I]hCG binding was found at 2 h ($P < 0.001$) after hCG injection. Consistent with earlier findings [21], binding activity fell within 7 h by about 75% and began to recover thereafter. Experiments with reconstitution of LH/hCG receptors into proteoliposomes from desensitized rat ovaries documented that 2 h after injection of hCG (onset of down-regulation) the receptor really disappeared and was not only masked, e.g. by aggregation or sequestration within endocytic vesicles (Fig. 2). The activity of the adenylylcyclase system in plasma membranes obtained from the rat ovary was not desensitized at 0.5 h after hCG injection, but the hormonal response was lost at 2 h (Fig. 3). However, hormone-responsive adenylylcyclase activity measured in the presence of GTP-γ-S was partially desensitized at 0.5 h after injection of the desensitizing dose of hCG (63% decline in the hCG-stimulated activity). On the other hand, luteal cells isolated from the rat ovary failed to respond not only at 2 h, but even at 0.5 h after hCG injection to hormonal stimulation by cAMP and progesterone production (Fig. 3). Similar results were found in progesterone secretion at 1.5 h in luteal cells from hCG-desensitized rats by Azhar et al. [22]. The loss of hormonal responsiveness of luteal cells after 0.5 h occurred without a reduction in the number of LH/hCG receptors (data not shown). Preincubation or extensive washing of luteal cells did not reverse the desensitized state. Moreover, basal production of cAMP and progesterone by luteal cells was appreciably elevated, which appears to be consistent with the characteristic pseudo-irreversible binding kinetics of the LH/hCG receptor [23].

The events implicated in the early process of desensitization may include changes in the order and dynamics of membrane lipids. The present studies indicate that ovarian membrane lipid rigidity decreased ($P < 0.001$) as early as 0.5 h after injection of the desensitizing dose of hCG and persisted for the following 50 h (Fig. 1). However, reincorporation of solubilized membrane components into the new lipid bilayer caused disappearance of differences in membrane lipid rigidity in desensitized luteal membranes (Fig. 2). The changes of the physical state of the membrane were also reflected in the information obtained by DSC (Fig. 4). Desensitization modified the calorimetric profile characteristic of the control sample. The main 40–53°C thermal transition became more expressive and cooperative and was shifted to lower temperatures. It is likely that this thermal transition reflects structural transitions of membrane phospholipids. The changes in the molecular order of membranes are closely correlated with differences in their cholesterol/phospholipid molar ratio. Under physiological conditions, a decrease of this ratio is associated with disordering of membrane lipids [24]. Therefore, the decrease in the rigidity

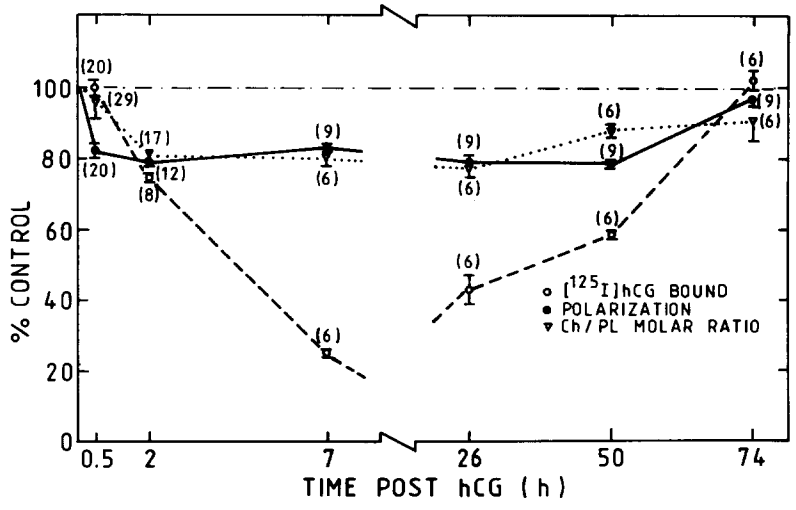

Fig. 1. Time course of changes in specific binding of [^{125}I]hCG (- - - -), cholesterol/phospholipid molar ratio (·····) and the degree of fluorescence polarization of DPH probe (——) in pseudopregnant rat ovaries following injection of 75 IU of hCG. Control values of binding were about 95 fmol hCG bound per mg protein. The figures in parentheses represent data pooled from 2–5 independent experiments. The means ± S.E.M. are shown.

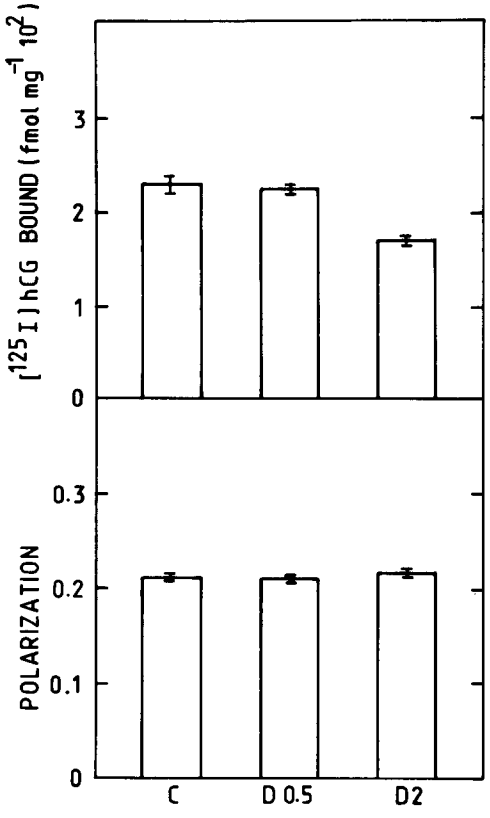

Fig. 2. Effect of a desensitizing dose of 75 IU of hCG injected 0.5 h (D 0.5) or 2 h (D 2) before sacrificing the rats, on the accessibility of the LH/hCG receptor and fluorescence polarization of the DPH probe in proteoliposomes with reconstituted receptor. The values are means ± S.E.M. of 6 determinations. The results were confirmed in 3 independent experiments.

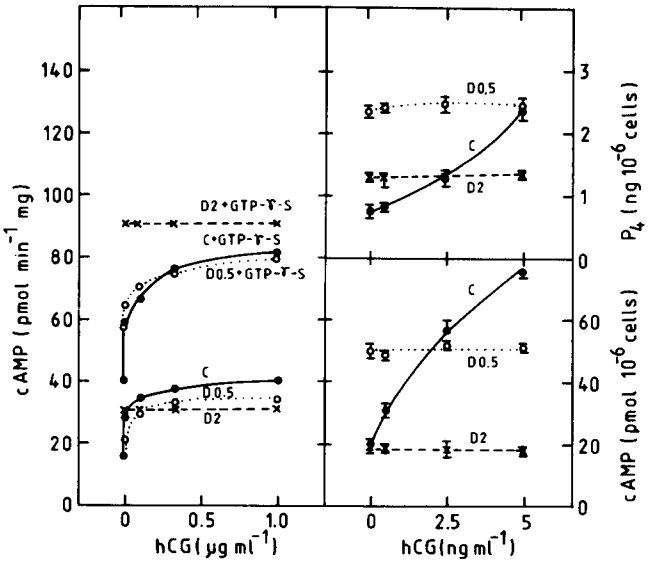

Fig. 3. hCG stimulated plasma membrane adenylylcyclase activity (left) and cAMP and progesterone (P_4) secretion by luteal cells (right) after injection of hCG. Isolated cells were prepared by collagenase digestion and after 30 min preincubation they were incubated for 3 h at 37°C. The rats were treated as described in the caption to Fig. 2. Each point is the means ± S.E.M. of 3–5 determinations. The experiments were repeated 3–4 times with similar results.

of membranes may be the result of an alteration in the cholesterol/phospholipid ratio; this was not the case in 0.5 h desensitization (Fig. 1). However, a fall in this ratio was observed at 2 h after injection of hCG, at the time when the cholesteryl ester content was found to be decreased in desensitized rat ovaries [22]. The change in membrane lipid fluidity was associated particularly with the process of desensitization of luteal tissue and with hCG treatment in vivo. Incubation of luteal membranes with hCG [25] or injection of male rats with a desensitizing dose of hCG [2] had no effect on ovarian or testicular membrane lipid fluidity. A rise in membrane disorders during insulin-induced receptor internalization (down-

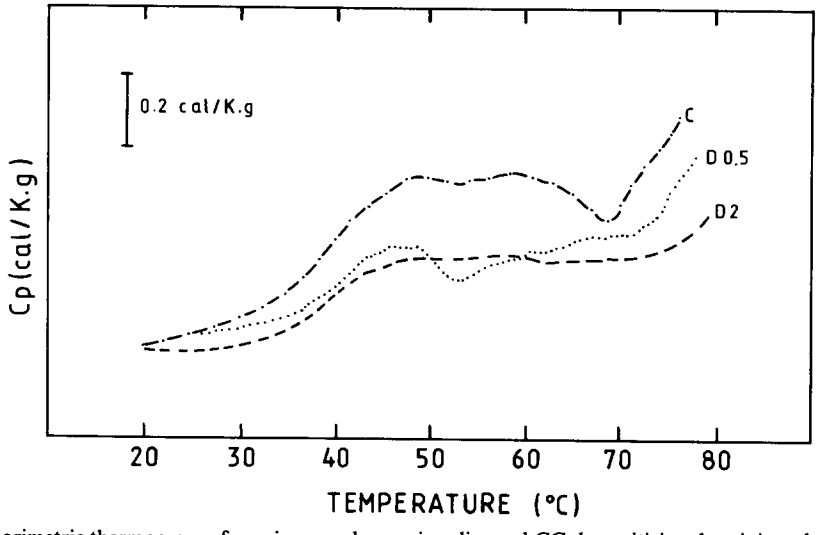

Fig. 4. Differential scanning calorimetric thermogram of ovarian membranes in saline or hCG desensitizing dose injected rats. The heat capacity was measured at protein concentration of about 1 mg · ml^{-1} in buffer A. Thermograms were highly reproducible. The rats were treated as described in the caption to Fig. 2.

regulation), which appears to be connected with concentrative endocytosis, was shown previously [12], but changes in membrane lipid fluidity in the early desensitization process have not been reported as yet. A positive correlation between elevation of rigidity of membrane lipids, LH/hCG receptors and steroidogenic systems was found during formation of rat corpora lutea [9]. On the other hand, a decline of membrane rigidity may be a requirement for induction of changes which lead to events of desensitization. Ekstrom and Hunzicker–Dunn [26] observed that ethanol, a well known lipid-fluidizing agent, potentiated the extent of hCG-induced desensitization of porcine luteal adenylylcyclases by intensifying an impairment of the coupling receptor with G_s proteins. Therefore, a decrease of membrane lipid rigidity, as an early effect of desensitization, may facilitate the transformation of the LH/hCG receptor to its desensitized state.

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