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Allopregnanolone and pregnanolone are produced by the human corpus luteum

Ulrika Ottander [a], Inger Sundström Poromaa [b,*], Erik Bjurulf [c], Åsa Skytt [d], Torbjörn Bäckström [a], Jan I Olofsson [e]

[a] *Department of Clinical Science, Obstetrics and Gynecology, Umeå University Hospital, Umeå, Sweden*
[b] *Department of Women's and Children's Health, University Hospital, 751 85 Uppsala, Sweden*
[c] *Department of Clinical Science, Division of Obstetrics and Gynaecology, Karolinska Institutet, Karolinska University Hospital Huddinge, Sweden*
[d] *Department of Medical Biosciences, Pathology, Umeå University, Sweden*
[e] *Fertility Center Scandinavia, Göteborg, Sweden*

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Abstract

Using a dispersed human luteal cell culture model, progesterone, allopregnanolone and pregnanolone release following treatment by incremental doses of human chorionic gonadotrophin (hCG) were evaluated. Corpus luteum tissues, obtained from 48 healthy women scheduled for benign surgery, were grouped according to luteal age and tissue concentration of allopregnanolone and pregnanolone was determined. The mRNA expression of 5α-, and 5β-reductase and 3α-HSOR mRNA expressions were evaluated in corpora lutea from the late luteal phase.

Allopregnanolone concentrations in corpus luteum tissue were consistently about three- to four-fold higher than pregnanolone levels. Allopregnanolone tissue concentrations significantly decreased between early- and late-luteal phase, $p < 0.05$. When exposed to hCG, progesterone output from freshly obtained human corpora lutea cells was two- three-fold increased compared to control levels. With 0.1 U/ml hCG a two-fold increase in allopregnanolone levels were noted, whereas pregnanolone levels were increased by approximately 40%. Furthermore, the mRNA of 5α-, 5β-reductase and 3α-HSOR mRNA were all expressed in human corpus luteum.

In conclusion, the neurosteroids allopregnanolone and pregnanolone are produced in the human corpus luteum and their release is stimulated by trophic hormone.

Keywords: Allopregnanolone; Human corpus luteum; Cell culture; 5β-Reductase

1. Introduction

Generally, steroid hormones exert their effects in the brain and other steroid sensitive target cells over a relatively long time by acting as transcription factors that regulate gene expression (McEwen, 1991). However, some of the effects of progesterone are mediated via its conversion to neuroactive metabolites (known as neuroactive steroids or neurosteroids) by several reductases and hydroxylases (Baulieu, 1991). When progesterone is processed by reductases either 5α-pregnene-3,20-dione (5α-DHP) is formed by 5α-reductase, or 5β-pregnene-3,20-dione (5β-DHP) by 5β-reductase. These products are further processed by 3α-hydroxysteroid oxidoreductase (3α-HSOR) to 3α-hydroxy-5α-pregnan-20-one (5α-THP/allopregnanolone) and 3α-hydroxy-5β-pregnan-20-one (5β-THP/pregnanolone) (Stoffel-Wagner, 2001).

Neurosteroids, such as allopregnanolone and pregnanolone, exert their actions through binding to the $GABA_A$ receptor, where they increase hyperpolarization and mediate anxiolytic (Wieland et al., 1991), anticonvulsant (Landgren et al., 1998) and sedative hypnotic effects (Sundström et

* Corresponding author. Tel.: +46 18 611 57 64; fax: +46 18 55 97 75.
E-mail address: inger.sundstrom@kbh.uu.se (I.S. Poromaa).

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al., 1998), in a manner similar to barbiturates and benzodiazepines (Lambert et al., 1995; Majewska et al., 1986).

Although there is evidence for de novo synthesis of neurosteroids in the human brain (Stoffel-Wagner, 2001), it is conceivable that progesterone, produced by the corpus luteum, is a significant source for neurosteroids in fertile women (Bäckström et al., 1986; Genazzani et al., 1998). Indirect evidence of synthesis of neuroactive steroids in the human corpus luteum can be drawn from the finding of a 22-fold increased concentration of 5α-DHP in the ovarian venous plasma draining the corpus luteum-containing ovary compared to concentrations found in plasma from the contralateral ovarian vein (Bäckström et al., 1986). There is also a close temporal relationship between peripheral progesterone and allopregnanolone concentrations during the luteal phase of fertile women (Wang et al., 1996). In certain mammals, isoallopregnanolone (3β-OH-5α-pregnan-20-one), 5α-DHP and allopregnanolone are all major biosynthesis products of the mammalian corpora lutea (Albert et al., 1982; Hodges et al., 1994, 1997; Lerner and Eckstein, 1976).

Furthermore, 5α-reductase, one of the enzymes needed for conversion of progesterone to allopregnanolone and pregnanolone, has previously been found in the human ovary (Haning et al., 1996). Other enzymes needed for formation of allopregnanolone and pregnanolone have not been investigated in the human corpus luteum. 5β-Reductase has previously only been thought to exist in the liver (Kondo et al., 1994) but has been reported to be expressed in the avian ovary (Gomez et al., 1998). The enzyme 3α-hydroxysteroid oxidoreductase, which further reduces 5α-DHP to allopregnanolone, has previously only been found in the rat ovary where its content varies with the stage of the estrous cycle (Takahashi et al., 1995).

However, in spite of this indirect evidence of neurosteroid synthesis in the human ovary, direct evidence for formation of allopregnanolone and pregnanolone in the human corpus luteum is lacking.

The purpose of the present study was to investigate the allopregnanolone and pregnanolone formation in the human corpus luteum using cell cultures from freshly obtained human corpora lutea. More specifically, the study aimed at evaluating the effect of hCG on luteal production of allopregnanolone pregnanolone in vitro, and the expression of 5α-reductase, 5β-reductase and 3α-HSOR mRNA in the human corpus luteum.

2. Material and methods

2.1. Patients

Forty-eight women were recruited in the study. All had given informed consent and the study was approved by the Ethical Committee of Umeå University Hospital. Ovarian tissue was obtained from women scheduled for laparotomy due to benign conditions (i.e. legal sterilization or uterine fibroma) at the Department of Obstetrics and Gynecology, Umeå University Hospital. The patients had not received any hormonal therapy during the preceding month and were otherwise healthy. The average age of the patients was 38.3 years (range 29–47). All women had proven fertility, and had a history of regular menstrual cycles ranging between 24 and 30 days. The corpus luteum age was determined according to onset of last period of menstruation and detection of an ovulatory LH-surge in urine (Clearplan One Step, Unipath Ltd., Bedford, UK). Day 1 was defined as the 1st day after a positive LH-test. According to these two parameters, the patients were scheduled for surgery in early (day 2–5) luteal phase, mid (day 6–10) or late (day 11–14) luteal phase. On the day of surgery a preoperative ultrasound was performed to localize the corpus luteum and blood samples were taken. During surgery the corpus luteum was extirpated and either immediately divided into pieces and placed in liquid nitrogen for further analysis or prepared for cell cultivation.

2.2. Luteal cell culture procedure

All cell culture reagents and drugs were purchased from Life technologies/Gibco BRL (Gaithersburg, MD, USA) unless otherwise specified. Freshly obtained corpus luteum tissues ($n = 5$, representing three samples from mid-luteal phase and two samples from late luteal phase) were immediately transported to the laboratory in ice-chilled incubation medium where the tissue was carefully minced and enzymatically dissociated in sterile filtered M199, containing 1.0 mg/ml Collagenase type V, 50 μg/ml DNAse 1, 1.5% BSA and 0.95 mM $CaCl_2$ (all from Sigma-Aldrich Corporation, St. Louis, MO, USA) during 40 min. The cell suspension combined with an equal volume of sterile saline (0.154 M) was layered onto 3.0 ml of a fixed Percoll gradient (density 1.117 g/ml) and centrifuged at $400 \times g$ for 40 min to remove blood cells and cellular debris. The enrichment of luteal cells was carefully collected from the interface, washed and resuspended in fresh M199 containing 26 mM $NaHCO_3$, 25 mM Hepes, 50 U/ml penicillin, 50 μg/ml streptomycin and 1% heat-inactivated fetal bovine serum. Cells were counted in a Bürker-chamber under a light microscope and the viability was in all experiments estimated to be above 90% by the trypan blue dye exclusion method. The volume of cell suspension was adjusted with M199 to give a concentration of 1.5×10^5 cells mL^{-1} medium and added to cell culture dishes (Nunclon, Nunc A/S Roskilde, Denmark) and preincubated at 37 °C in humidified air/5% CO_2 for 18–24 h in a Forma-Scientific CO_2 incubator, model 3196 (Marietta, OH, USA). Following the change to fresh medium, adherent cells were treated by incremental doses of hCG (Profasi®, Ares-Serono S.A. Geneva, Switzerland), in triplicate wells. The concentration used in the dose response for hCG ranged from 0.0001 to 1.0 U/ml. Cultures were terminated after 24 h and the medium was collected and stored at −20 °C until assayed.