Dominant expression and distribution of oestrogen receptor β over oestrogen receptor α in the human corpus luteum

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To investigate the potential importance of oestrogen as a local regulator of human corpus luteum function, the mRNA expression pattern and cellular localization of oestrogen receptors (ERs), ER-α and ER-β, were studied in corpora lutea grouped according to age, where days 2–5 post-LH rise were designated as the early luteal phase, days 6–10 as mid-luteal and days 11–14 as the late luteal phase respectively. Northern blot analysis using an ER-β probe in samples from whole ovarian tissue and isolated corpora lutea, revealed a major band at 7.5 kb and several minor bands between 4–10 kb, while no signals for ER-α mRNA were obtained. However, using a semi-quantitative reverse transcription–polymerase chain reaction followed by Southern blotting, ER-β mRNA levels were found to be 63% lower (P < 0.05, n = 39) in the mid-luteal phase compared with the early luteal phase, while ER-α mRNA expression showed no statistical differences between the different age groups. Using in-situ hybridization, ER-β mRNA expression was localized to the steroidogenic luteal cells as well as perivascular cells and fibroblasts in the corpus luteum. Immunohistochemistry confirmed the localization of ER-β protein, but no clear staining of luteal cells was found using antibodies against ER-α. Collectively, the findings of low to moderate expression of ER-β mRNA and protein in the steroidogenic cells, and also in vascular endothelial cells of the corpus luteum, as opposed to diminutive amounts of ER-α mRNA, suggest that oestrogen activity is primarily transduced via ER-β in the human corpus luteum.

Key words: 17β-oestradiol/human corpus luteum/oestrogen receptors/steroids

Introduction

The luteal autocrine/paracrine system features a functional core primarily composed of two steroidogenic cell types distinguished by their size, commonly denoted as large and small luteal cells. These cells appear to be in close interaction with a heterogeneous satellite population of non-steroidogenic cell types, e.g. vascular endothelial cells, perivascular fibroblasts, macrophages, and various immunoregulatory cells. Only recently, these cell types have been attributed to exert physiologically important roles (Nappi et al., 1994; Brännström and Friden, 1997; Gaytan et al., 1999). Oestrogen and progesterone mediate their actions through specific intracellular receptors that act as hormone-dependent transcriptional regulators (Beato et al., 1995), and are thought to be intrinsically involved in the intraovarian modulatory system. Several studies have indicated that progesterone acts as a luteotrophin that promotes its own synthesis and maintains the structural integrity of the corpus luteum (Rothchild, 1981; Duffy et al., 1994). In monkey and human corpus luteum, these effects appear to be transmitted through progesterone receptors (PRs) which are predominantly localized in the steroidogenic cells (Chandrasekher et al., 1994; Ottander et al., 2000).

Oestrogens have also been postulated as a factor that modulates corpus luteum function, however, no universal functional role of intraluteal oestrogen action can be discerned across the species. In species such as rabbit, rat and pig, oestrogen is luteotrophic (Niswender et al., 2000), as opposed to monkey and human, where oestrogen seems to act in a luteolytic mode (Gore et al., 1973; Vega et al., 1994; Duffy et al., 2000). The human oestrogen receptor (ER) is expressed in at least two subtypes, ER-α (Greene et al., 1986) and ER-β (Mosselman et al., 1996). These genes are mapped to chromosome 6q25.1 and 14q22-24 respectively, and several splice variants of both ER-α (Zhang et al., 1996) and ER-β (Moore et al., 1998) have been identified. The binding affinities of ER-α and ER-β to 17β-oestradiol are almost identical,
possibly due to a high degree of homology in the hormone binding domains (58% amino acid sequence homology) and in the DNA binding domain (96% amino acid sequence homology) (Mosselman et al., 1996).

In a limited number of studies, ER-α expression in the human corpus luteum has been demonstrated (Revelli et al., 1996; Misao et al., 1998, 1999), while other studies have raised questions about whether ER-α is expressed in the corpus luteum of primates (Iwai et al., 1990; Chandrasekher et al., 1994; Suzuki et al., 1994). The presence of ER-β in the human corpus luteum has recently been reported (Misao et al., 1999; Taylor and Al Azzawi, 2000), but the expression pattern during different developmental stages and cellular localization has not been fully characterized.

In the present study, we have investigated both the expression and localization of ER-α and ER-β mRNA and protein during different developmental stages of the human corpus luteum.

Materials and methods

Patients

A total of 41 women were recruited. All had given informed consent and the study was approved by the Ethical Committee of Umeå University Hospital. Ovarian tissue was obtained upon commencing elective surgery due to benign conditions (i.e. legal sterilization, n = 26 or uterine fibroma, n = 15) at the Department of Obstetrics and Gynecology, Umeå University Hospital, Sweden. The patients had not received any hormonal therapy during the preceding month and were otherwise healthy. The average age of the patients was 38.2 ± 0.8 years (range 29–47). All patients had proven fertility, and had a history of regular menstrual cycles of 24–30 days. According to the onset of the last period of menstruation, detection of an ovulatory LH surge in urine (Clearplan One Step; Unipath Ltd, Bedford, UK) was performed using the appropriate RNA polymerase in an in-vitro transcription system (Promega) with [α-32P]-labelled UTP (800 Ci/mmol; Amersham, Buckinghamshire, UK) for subsequent Northern blot analysis, or using a DIG RNA labelling kit (SP6/T7) (Roche Diagnostics, Basel, Switzerland) with digoxigenin-labelled UTP for subsequent in-situ hybridization.

Hormone assay

Pieces of corpus luteum tissue were weighed (33.6 ± 3.3 mg, n = 26) and placed in 95% v/v ethanol for 48 h at +4°C in thoroughly sealed tubes according to a previously described procedure (Ottander et al., 2000). Concentrations of 17β-oestradiol in corpus luteum tissue and serum were determined by a time-resolved fluorometric assay (Delfia; Wallac Ltd, Turku, Finland), according to the manufacturer’s protocol. All samples were analysed in duplicate in a single assay, where the coefficient of variation was <5%.

RNA extraction

Corpora lutea tissues were homogenized using a mikrodissembrator (model U; Braun Biotech International, Melsungen, Germany) in guanidine isothiocyanate solution, and total RNA was isolated using CsCl gradient ultra-centrifugation and phenol/chloroform extraction (Glisin et al., 1974; Chomczynski and Sacchi, 1987). The total RNA concentration was determined by spectrophotometry (Lambda2 UV/VIS spectrophotometer; Perkin-Elmer, Überlingen, Germany), and integrity of total RNA samples was verified by 1% agarose gel electrophoresis in the presence of ethidium bromide followed by visualization under UV light (312 nm; Spectroline model TVL-312A; Spectronics Corporation, Westbury, NY, USA). For Northern blot analysis, poly(A)+ RNA was recovered from the total RNA of pooled corpora lutea or whole ovarian tissue using a PolyA Tract mRNA isolation systems III (Promega, Madison, WI, USA) according to the manufacturer’s protocol.

Synthesis of cRNA probes

Polymerase chain reaction (PCR) fragments of ER-α cDNA (292 bp; nucleotides 1310–1601) (Greene et al., 1986) and ER-β cDNA (358 bp; nucleotides 104–461) (Mosselman et al., 1996) were subcloned into plasmid vectors (pCRII-TOPO TA cloning kit; Invitrogen, BV, Leek, The Netherlands). Prior to transcription, plasmids were purified (QIA-prep spin miniprep kit; Qiagen GmbH, Hilden, Germany) and linearized with suitable restriction enzymes, so that antisense or sense RNA probes could be obtained. Transcription was performed using the appropriate RNA polymerase in an in-vitro transcription system (Promega) with [α-32P]-labelled UTP (800 Ci/mmol; Amersham, Buckinghamshire, UK) for subsequent Northern blot analysis, or using a DIG RNA labelling kit (SP6/T7) (Roche Diagnostics, Basel, Switzerland) with digoxigenin-labelled UTP for subsequent in-situ hybridization.

Semi-quantitative reverse transcription–polymerase chain reaction (RT–PCR)

A previously validated semi-quantitative RT–PCR method (which involves co-amplification of the target sequence and an endogenous control sequence whose content is not altered by the experimental manipulation) was used to correct for variable product yields (Innis et al., 1995; Saric and Shain, 1997). This method was employed to quantify the mRNA expression ratio of either ER-α or ER-β against ubiquitously expressed glyceraldehyde-3-phosphate dehydrogenase (GAPDH; Ottander et al., 1999) in corpus luteum tissue. For reverse transcription, a Ready-To-Go T-Primed First-Strand Kit (Amersham Pharmacia Biotech Inc., Uppsala, Sweden) was used with 2.0 μg of each total RNA sample, to synthesize cDNA which was then stored at −20°C until PCR was carried out. A multiplex PCR with simultaneous amplification of GAPDH and either ER-α or ER-β cDNA in the same tube, was carried out in the presence of 1 μl of cDNA aliquot, 2.5 mmol/l dNTP mixture, PCR buffer (10 mmol/l Tris–HCl; pH 8.3, 50 mmol/l KCl, 1.5 mmol/l MgCl2, 0.01% w/v autoclaved gelatin), 0.625 IU Taq DNA polymerase (Roche Diagnostics, Basel, Switzerland), 0.2 μmol/l GAPDH-sense/antisense primers and either 0.2 μmol/l ER-α-sense/antisense primers or 0.2 μmol/l ER-β-sense/antisense primers (Table I), in a total volume of 20 μl using a PC-960G Gradient Thermal Cycler (Corbett Research, NSW, Australia). Each PCR consisted of 24 cycles (30s at 94°C, 30s at 60°C, 45s at 72°C) followed by 10 min at 72°C. PCR products were then separated on 1.5% agarose gels by electrophoresis, transferred onto Hybond-N nylon filters by capillary transfer, cross-linked by UV irradiation and subjected to standard Southern analysis using fluorescein-labelled internal cDNA probes. To prepare these cDNA probes, each cDNA specific sequence fragment (i.e. ER-α, ER-β and GAPDH; see Table I) subcloned in pCRII-TOPO plasmids, was amplified for labelling with PCR fluorescein labelling mix (Roche Diagnostics, Basel, Switzerland). The filters were prehybridized in 5× sodium chloride/sodium citrate (SSC), 0.1% sodium dodecyl sulphate (SDS), 5% dextran sulphate, 20-fold dilution of liquid blocking agent (RPN3601; Amersham Pharmacia Biotech Inc, Uppsala, Sweden) and 100 μg/ml heat-denatured herring sperm DNA at 60°C.
Table I. Position and sequence of primers and number of cycles used for polymerase chain reaction (PCR) amplifications of oestrogen receptor (ER-α, ER-β) and GAPDH cDNAs and position of internal control plasmid Southern probe

<table>
<thead>
<tr>
<th>Gene</th>
<th>Outer PCR primer sequence (bp)</th>
<th>Inner PCR primer sequence (bp)</th>
<th>Product size (bp)</th>
<th>No of cycles</th>
<th>Control plasmid Southern probe size (bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ER-α</td>
<td>5'-CACCTGGGCACCTTTCTCCTTTAG</td>
<td>5'-CAGCTCTTGCGCCGGTTTTTATC</td>
<td>439</td>
<td>24</td>
<td>513</td>
</tr>
<tr>
<td>ER-β</td>
<td>5'-GATGCCCCTCCACGGCTAGT</td>
<td>5'-GATGCCCCTCCACGGCTAGT</td>
<td>707/523</td>
<td>24</td>
<td>386</td>
</tr>
<tr>
<td>GAPDH</td>
<td>5'-AAGGTCGGAGTCAACGGATT</td>
<td>5'-CATGAGTCCTTCCACGATAC</td>
<td>539</td>
<td>24</td>
<td>57</td>
</tr>
</tbody>
</table>

The membranes were washed with 5× SSC for 5 min and the same procedure was repeated for GAPDH detection. The autoradiograms were scanned for intensity of hybridization using a computerized densitometer (Fast Scan-Computing Densitometer, series 300; Molecular Dynamics, Baltimore, MD, USA), and the arbitrary densitometric level of respective signal was determined and analysed by ImageQuant (Software version 3.22; Molecular Dynamics).

Sequence analysis

All the PCR products were subcloned into pCRII-TOPO vector and subjected to sequence analysis (Thermo Sequence dye terminator cycle sequencing pre-mix kit; Amersham Life Science Inc, Cleveland, OH, USA) by an automatic laser cycle sequencing apparatus (377 DNA sequencer, GeneScan software; Perkin-Elmer) according to the manufacturer’s instructions.

Northern blotting

Poly(A)+ messenger RNA samples (5.0 µg/well) were size-fractionated by 1% agarose gel electrophoresis in the presence of formaldehyde (Sambrook et al., 1989), transferred onto nylon filters (Hybond-N; Amersham) by capillary transfer, and cross-linked by UV irradiation. The filters were prehybridized in 50% formamide, 750 mmol/l NaCl, 15 mmol/l sodium citrate, 8× Denhardt’s solution (1.6 mg/ml Ficoll, 1.6 mg/ml polyvinylpyrrolidone, 1.6 mg/ml bovine serum albumin), 0.1% SDS, 10 mmol/l EDTA, 25 mmol/l Tris–HCl; pH 7.0, 250 µg/ml heat-denatured herring sperm DNA and 250 µg/ml yeast tRNA at 62°C for 2 h. Hybridization was carried out in the same solution containing 2×10⁶ cpm/ml each cRNA probe for either ER-α or ER-β for 16 h at 64°C. After hybridization, the filters were washed twice in 2× sodium chloride/sodium citrate (SSC) and 0.1% SDS for 15 min at 20°C, followed by two washes in 0.1× SSC and 0.1% SDS for 40 min at 66°C. The results of autoradiography were visualized by a PhosphorImager (Molecular Dynamics, Sunnyvale, CA, USA) and analysed using Molecular Analyst version 1.4 software (Bio-Rad Laboratories, Hercules, CA, USA).

In-situ hybridization

The in-situ hybridization was performed essentially as previously described (Panoksalinis-Mortari and Bucy, 1995). Briefly, 7 µm cryostat sections were collected on SuperFrost/Plus slides (Menzel-Glaser, Brann Scheig, Germany), fixed in 3% paraformaldehyde (in diethylypyrocarbonate (DEPC)-treated phosphate-buffered saline) at room temperature for 1 h, rinsed with 0.1 mol/l tricholamine (pH 8.0) and acetylated with 0.25% acetic anhydride for 15 min. Hybridization was performed in a solution of 50% formamide, 10% dextran sulphate, 4× SSC, 5× Denhardt’s solution, 250 µg/ml yeast tRNA, 500 µg/ml herring sperm DNA and 1 µg/ml digoxigenin-labelled antisense or sense ER-α and ER-β riboprobe overnight at 50°C. After hybridization, the slides were incubated in RNase solution (40 µg RNase A/1ml STE (500 mmol/l NaCl, 20 mmol/l Tris–HCl; pH7.5, 1 mmol/l EDTA) buffer) for 30 min at 37°C followed by washing.
incubation in blocking buffer (2% normal horse serum, 100 mmol/l Tris–HCl; pH 7.5, 150 mmol/l NaCl). Alkaline phosphatase labelled sheep-anti-digoxigenin antibodies (diluted 1:100 in blocking buffer; Roche Diagnostics, Basel, Switzerland) were added and the slides incubated at room temperature for 1 h. Afterwards, substrate solution (337.5 µg/ml 4-Nitroblue Tetrazolium chloride, 175 µg/ml 5-bromo-4-chloro-3-indolyl-phosphate toluidinium salt, 100 mmol/l Tris–HCl; pH 9.5, 100 mmol/l NaCl, 50 mmol/l MgCl₂) was added and slides were incubated in the dark at 4°C for 3 h overnight before the colour reaction was terminated in stopping buffer (10 mmol/l Tris–HCl; pH 8.0, 1 mmol/l EDTA). All hybridization and incubation steps were performed in humid chambers. Results were evaluated by light microscopy and slides were documented by photography.

**Immunohistochemistry**

Corpora lutea were immersion-fixed in Bouin’s solution for 4 h, dehydrated and embedded in paraffin. Sections (4 µm thick) mounted on glass slides were stained with haematoxylin and eosin. Adjacent mounted sections were deparaffinized, rehydrated, immersed in citrate buffer (10 mmol/l, pH 6.0) and heated in a microwave oven (600 W) for 3×5 min as earlier described (Shi et al., 1991). The localization of antigen–antibody complexes was performed using the avidin–bixin–peroxidase complex (ABC) technique (Hsu et al., 1981) using a Vectastain avidin–bixin–peroxidase kit (Vector Laboratories, Burlingame, CA, USA). The slides were immersed in 3% H₂O₂ in methanol (30 min) to suppress endogenous peroxidase activity. Thereafter, 2×5 min rinses in buffer followed by 20 min preincubation in buffer containing 3% normal goat serum (NGS) were carried out to decrease non-specific background staining. The sections were incubated overnight at 4°C with the affinity-purified, polyclonal primary antibody raised against ER-β (1 µg/ml Erβ 14-A in buffer supplemented with 3% NGS; Alpha Diagnostic International, San Antonio, TX, USA). The sections were incubated in chronological order with a biotinylated secondary antibody (goat anti-rabbit immunoglobulin G; Vector Laboratories) for 30 min, with ABC reagents for 30 min and with peroxidase substrate (0.03% dianaminobenzidine tetrahydrochloride and 0.015% H₂O₂ dissolved in buffer) for 15 min. Between and after incubations, the sections were washed twice for 5 min each in buffer. The sections were counter-stained with Mayer’s haematoxylin followed by routine dehydration and mounting with coverslips. Three different control incubations with buffer, with non-immune serum and with immune serum and added control peptide (Human Erβ 14P; Alpha Diagnostic International, San Antonio, TX, USA) diluted together with the primary antibody according to the manufacturer’s recommendations were negative. Moreover, validation experiments with two additional antibodies for ER-β, PAI-311 (SDS, Falkenberg, Sweden) and ER-β 503 (KarloBio, Huddinge, Sweden) were performed with the same method. For immunohistochemical detection of ER-α, using an ER-α antibody (M 7047) (DACO, Glostrup, Denmark) the same methodology was employed.

**ER-α protein assay**

Frozen corpus luteum tissue was homogenized and suspended in cold standard receptor buffer (10 mmol/l Tris pH 7.4, 1.5 mmol/l EDTA, 10 mmol/l sodium molybdate, 1.0 mmol/l monothioglycerol). Supernatants were collected after 10 min of refrigerated centrifugation at 20,000 g and used for receptor detection. The pellet fractions were analysed for DNA content by the diphenylamine method (Burton, 1968) in order to evaluate cell concentrations in sample. Detection of total cellular ER-α protein was attempted by enzyme immunoassay (ELA) kit (Abbott Laboratories, North Chicago, IL, USA) according to the manufacturer’s instructions. Receptor concentration was expressed as femtomol receptor/µg DNA, where the assay sensitivity was ≥0.1 femtomol/µg DNA.

**Figure 1.** 17β-oestradiol concentrations in serum and corpus luteum tissue, detected by time-resolved fluorometric assay (Delfia) during early (days 2–5, n = 13), mid- (days 6–10, n = 15) or late (days 11–14, n = 13) luteal phase. The Mann–Whitney U-test was used to test differences between groups.

**Statistical analysis**

All the experiments for Northern blot analyses, RT–PCR/Southern blot analyses, in-situ hybridization, immunohistochemistry and hormone assay were repeated at least three times with similar results obtained. Values are given as mean ± SEM. Differences between groups were tested using the non-parametric Mann–Whitney U-test. Spearman’s non-parametric rank correlation test was employed when showing a correlation between two groups. P < 0.05 was considered to be statistically significant.

**Results**

The concentrations of 17β-oestradiol in corpus luteum tissue and peripheral serum during the three different post-ovulatory stages were determined. As shown in Figure 1, the lowest tissue concentrations were found during the late luteal phase, whereas peripheral serum 17β-oestradiol did not vary significantly during the different luteal phases.

To determine the expression ratio of ER-α and ER-β mRNA, a multiplex semi-quantitative RT–PCR followed by Southern blot analysis was employed. Using this highly sensitive method, ER-α mRNA and ER-β mRNA could be amplified and adjusted according to the amplification of GAPDH mRNA (Figure 2A,B). When the ER-α primers were used, two clear fragments, which both hybridized to the internal cDNA probe, were amplified. Sequence analysis revealed that the large fragment (707 bp) was the full-length ER-α mRNA and the short fragment (523 bp) originated from an alternatively-spliced variant mRNA lacking exon 7 (SV7 ER-β, McGuire et al., 1991). The ratio between SV7 ER-α mRNA expression and non-spliced ER-α mRNA showed no significant variation between the different luteal phase groups (data not shown).

Although mid-luteal corpora lutea tended to show a lower expression of ER-α mRNA compared with the other luteal phases, no statistically significant differences were found between the three groups (Figure 2C). On the other hand, ER-β mRNA expression was markedly lower (reduced to 37%), in the mid-luteal phase compared with the early luteal phase (P < 0.05), while no significant changes between the late luteal phase and the earlier stages were noted (Figure 2D;
**Human corpus luteum oestrogen receptor distribution**

Figure 2. Semi-quantitative reverse transcription–polymerase chain reaction (RT–PCR)/Southern blot analysis of oestrogen receptor α (ER-α) and oestrogen receptor β (ER-β) mRNA expression in human corpus luteum of the menstrual cycle. The internal standard glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was co-amplified. (A) Representative blot of ER-α and GAPDH and (B) ER-α and GAPDH. (C) Also shown is the ratio of ER-α mRNA and GAPDH mRNA expression and (D) ratio of ER-β and GAPDH mRNA expression during different age periods of the human corpus luteum (total \( n = 38 \); early \( n = 11 \), mid- \( n = 15 \), late \( n = 12 \)). The Mann–Whitney U-test was used to test differences between groups.

\( n = 11 \) early luteal phase, \( n = 15 \) mid-luteal phase, \( n = 13 \) late). When considering a putative relationship in the level of expression between the two ER genes, it is interesting to note that a high degree of correlation (\( n = 39 \), \( r = 0.74 \), \( P < 0.001 \)) between ER-α and ER-β mRNA expression was found. When analysing the relationship between ER mRNA expression and 17β-oestradiol concentration in tissue and serum, the mRNA expression levels and concentration of 17β-oestradiol in corpus luteum tissue were found to be inversely correlated both for ER-α and ER-β mRNA (ER-α; \( r = -0.45 \), \( P < 0.01 \) and ER-β; \( r = -0.31 \), \( P < 0.05 \); \( n = 39 \)). However, no statistically significant correlations between the respective ER mRNA levels and serum 17β-oestradiol concentrations were noted (data not shown).

Northern blot analysis using a \([^{32}P]\)-radiolabelled ER-β cRNA probe and 5.0 µg of poly(A)+ selected RNA from corpus luteum tissue and whole ovarian tissue was performed. As shown in Figure 3, multiple transcripts were detected in both whole ovarian tissue and corpus luteum tissue, although hybridization was somewhat stronger in whole ovary than in corpus luteum. The most intense signal appeared at 7.5 kb, in agreement with a previous study (Mosselman et al., 1996), but additional signals at 10, 9 and 6 kb, were also observed. No hybridization signals could be visualized for ER-α mRNA either in the samples from corpus luteum or whole ovary (data not shown).

In an attempt to obtain more detailed information on the cellular localization of the ERs, in-situ hybridization and immunohistochemistry were performed. When an ER-β probe was hybridized to sections of frozen tissue, a clear positive signal for ER-β mRNA was seen in the steroidogenic cell-layer. ER-β mRNA was also detected in some, but not all, stromal cells, vascular endothelial cells and spindle-shaped fibroblast-like cells in the perivascular region (Figure 4A–C).

Localization of ER-β antigen by immunohistochemistry using several different antibodies with similar results, confirmed the in-situ hybridization data. As shown in Figure 5A–C a clear cytoplasmic staining was obtained. No staining was revealed when the ER-β-specific antiserum was co-incubated with the peptide used for the immunization (Figure 5D). More prominent staining in the steroidogenic luteal cells, when compared with that observed by in-situ hybridization, was seen (Figures 4A,B and 5A–C). No clear immunolocalization of ER-α to luteal cells was detected when using an anti-ER-α.
antibody in corpora lutea tissues (Figure 6). Moreover, using an EIA for quantification of ER-α receptor concentration, levels were below the threshold level of detection in all corpora lutea tested (n = 24, data not shown).

**Discussion**

In this study, the mRNA expression patterns and distribution for both known subtypes of ERs, ER-α and ER-β, in the human corpus luteum of the menstrual cycle, were determined. In addition, the cellular and subcellular localization of ER-β protein in the corpus luteum has been described. Both serum and tissue concentrations of 17β-oestradiol were also measured. These data provide indications of the functional role of oestrogen action in the human corpus luteum. Tissue samples were obtained from prospectively recruited, regularly cycling, fertile, hormonally-untreated and otherwise healthy women. A high precision in establishing the age of the corpus luteum was secured by detection of a preovulatory LH surge, rather than by retrospective dating through arbitrary evaluation of endometrial histology (Dawood, 1994).

A disparity between the serum and corpus luteum tissue concentrations of 17β-oestradiol was found in later developmental stages of the corpus luteum. The decrease in tissue concentration could depict the commencing functional luteal regression, as this is likely to involve a lowered luteal synthesis of 17β-oestradiol. Alternatively, an increased metabolism and/or impaired influx of 17β-oestradiol derived from other sources (e.g. ovarian follicles), due to regressive changes in the microvasculature (Gaytan et al., 1999) may also be underlying causes.

Expression of ER-β mRNA could be detected by all methods which were employed (i.e. Northern blots, RT-PCR/Southern blot analysis and in-situ hybridization). The relative levels of ER-β mRNA exhibited an interesting dynamic shift during corpus luteum development; being high in the early luteal phase, low in the mid-luteal phase and intermediate in the late luteal phase. This finding contrasts with that of a previous report (Misao et al., 1999) in which decreasing amounts of ER-β mRNA with the age of the corpus luteum, were found. Differences in mRNA quantification methods, patient characteristics and dating of the corpora lutea may account for these, as yet unresolved, discrepancies. In support of these findings, increased ER-β mRNA expression in monkey corpora lutea in the late luteal phase, has recently been reported (Duffy et al., 2000).

Interestingly, the relative levels of each ER mRNA were found to be inversely (albeit weakly) correlated with the corpus luteum tissue 17β-oestradiol concentration. From these results, it may be postulated that 17β-oestradiol exerts a negative influence over ER-β gene expression in the human corpus luteum. This effect may be tissue-specific, since in human breast cancer cells, ER-β levels are up-regulated by oestradiol (Vladusic et al., 2000), while in rat granulosa cells, oestradiol treatment decreases ER-β binding activity and immunoreactivity (Sharma et al., 1999). In addition to direct effects by
Figure 5. (A–C) Immunohistochemical detection of oestrogen receptor β (ER-β) in a day 6 corpus luteum after incubation with the polyclonal primary antibody (Erb 14-A) and (D) immune serum added with peptide used for immunization (human Erb 14 control peptide), diluted together with the polyclonal primary antibody (Erb 14-A). Specific cytoplasmatic staining (brown) was located in the steroidogenic cells (arrowheads) and in some of the stromal cells (arrows), as well as in the endothelial cells (arrowheads) and in perivascular cells (arrows). The results are representative of a total of 20 corpora lutea studied. Original magnification (A, C, D) ×200 and (B) ×400.

Figure 6. Immunohistochemical detection of oestrogen receptor α (ER-α) in a human Fallopian tube and a day 12 corpus luteum after incubation with the monoclonal antibody (M7047). (A) Positive staining (brown, indicated by arrows) was located to the epithelium of the Fallopian tube; original magnification ×400 (B) while no positive staining was observed in the corpus luteum; original magnification ×400.

17β-oestradiol, it is also possible that the high numbers of progesterone receptors in the mid-luteal phase corpus luteum might transduce a progesterone-mediated down-regulation of ER-β mRNA expression (Ottander et al., 2000). To this end, it has recently been demonstrated in the monkey corpus luteum, that the loss of progesterone action increases ER-β mRNA levels during the mid-luteal phase (Duffy et al., 2000).

In-situ hybridization analysis showed that ER-β mRNA is
expressed in stromal cells, particularly perivascular stromal cells and steroidogenic luteal cells. This mRNA localization was in good agreement with the predominant immunohistochemical staining of both large and small luteal cells, comprising the steroidogenic layer. In addition, specific immune staining was seen in perivascular fibroblasts and the endothelial cells of the corpus luteum. This staining pattern, with a cytoplasmatic dominance, is consistent with a recent report of immunohistochemical localization of ER-β in the human corpus luteum (Taylor and Al-Azzawi, 2000).

Our attempts to detect ER-α protein were unsuccessful, and ER-α mRNA could only be detected by using RT–PCR amplification in combination with Southern blot analyses. Interestingly, this method disclosed two clear bands, which upon sequence analysis were found to be the full-length ER-α mRNA and the exon 7-lacking splicing variant (SV7) ER-α mRNA (McGuire et al., 1991). Previously, it has been reported from breast cancer tissues, that SV7 ER-α mRNA might inhibit the binding of full-length ER-α mRNA to its cognate response element and interfere in a dominant negative manner with fully processed ER-α function thereby playing an important physiological or pathophysiological role in vivo (Fuqua et al., 1992). Whether such a mechanism is operative in the human corpus luteum, remains unanswered, however, no changes in the relative expression ratio of full-length against spliced corpus luteum, remains unanswered, however, no changes in cytoplasmatic dominance, is consistent with a recent report of cells of the corpus luteum (Taylor and Al-Azzawi, 2000). This staining pattern, with a corpus luteum (Taylor and Al-Azzawi, 2000).

Taken together, our findings support the concept of a autocrine/paracrine role of oestrogen, primarily transduced via ER-β, in the human corpus luteum of the menstrual cycle.

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References

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