Endometrial nuclear receptor co-factors SRC-1 and N-CoR are increased in human endometrium during menstruation

F.Wieser1, C.Schneeberger1, G.Hudelist2, C.Singer2, C.Kurz1, F.Nagele1, C.Gruber1, J.C.Huber1 and W.Tschugguel1,3

1Division of Gynaecological Endocrinology and Reproductive Medicine and 2Division of Special Gynaecology, Department of Obstetrics and Gynaecology, University of Vienna Medical School, Vienna, Austria

3To whom correspondence should be addressed. E-mail: walter.tschugguel@akh-wien.ac.at

Steroid hormone receptor co-factors are abundantly expressed in the uterus in order to modify steroid hormone receptor action, either leading to activation or repression of transcription in the endometrium. However, the role of co-factors in remodelling of the human endometrium has not been established. We therefore endeavoured to evaluate the presence of the co-activator SRC (steroid receptor co-activator)-1 and the co-repressors N-CoR (nuclear receptor co-repressor) and steroid co-repressor SMRT (silencing mediator of retinoid and thyroid) receptors in the human endometrium during the different phases of the menstrual cycle. By using a real-time RT-PCR assay, we showed that SRC-1, N-CoR and SMRT mRNA are expressed in human endometrium during all phases of the menstrual cycle, as well as in inactive endometrium. Moreover, endometrial expression of SRC-1 and N-CoR mRNA increased during menstruation when compared with the other phases of the menstrual cycle (P < 0.001). Immunohistochemistry demonstrated that SRC-1 and N-CoR stain positive in the glandular epithelium and stroma in menstrual phase endometrium. The staining was weak in proliferative and secretory endometrium and absent in inactive endometrium. Our results suggest that differential expression of endometrial steroid receptor co-factors probably play a role in the regulation of human endometrium remodelling.

Key words: endometrium/menstrual cycle/N-CoR/SMRT/SRC-1

Introduction

In the human menstrual cycle, the endometrium undergoes a regular sequence of proliferation, differentiation and degeneration in response to the fluctuation of steroid hormone levels (Irwin et al., 1991). Steroid hormones are known to exert their effects on cell growth, development and differentiation in the human endometrium through intracellular steroid hormone receptors (SHRs), which are present in endometrial epithelium and stroma (Lessey et al., 1988; Tseng and Zhu, 1997; Matsuzaki et al., 1999). Several models have been proposed to explain the exact mechanisms by which ligand binding to SHRs activates gene transcription in hormone responsive tissues such as the endometrium and the breast (Thenot et al., 1999; Fu et al., 2000; Wang et al., 2000). Unliganded SHRs are attached to receptor-associated proteins termed heat shock proteins, which stabilize the receptor in an inactivated state. The binding of steroids to SHRs leads to dissociation of heat shock proteins, receptor dimerization, phosphorylation and binding of the activated SHR to hormone response elements (HREs) in the promoter region of target genes. The ligand–SHR complexes activate or repress gene transcription machinery through protein–protein interaction with other sequence-specific transcription factors or chromatin factors, or cross-talk with other signal transduction pathways (Beato and Klug, 2000; Nilsson et al., 2001). Katzenellenbogen and O’Malley proposed a tripartite model (ligand–receptor–co-factor) with liganded steroid receptors working through complexing proteins, i.e. hormone co-activators enhancing transcription (Katzenellenbogen et al., 1996).

A growing number of co-factors have been identified to mediate initiation (co-activators) or repression (co-repressors) of target gene transcription mediated by nuclear receptors in several organs (Horwitz et al., 1996; Torchia et al., 1998). In terms of transcriptional activation of estrogen receptors, the 160 kDa steroid receptor co-activator proteins which include SRC (steroid receptor co-activator)-1 and the p300/CPB (cAMP-response-element binding proteins) (Smith et al., 1996; Beato and Klug, 2000) are the most important. The mechanisms of co-factors imply several modes of action. SRC-1 enhances stabilization of the pre-initiation complex by direct or indirect interaction with general transcription factors (Liu et al., 1999). A second activating mechanism of SRC-1 is that it displays histone acetylase activity (Sterner and Berger, 2000). Acetylation of histones is thought to remodel the chromatin, stabilize the pre-initiation complex, and hence facilitate transcription.

In contrast, the repression of transcription is yielded by association of agonist- or antagonist-occupied receptors with co-repressors, including N-CoR (nuclear receptor co-repressor) and SMRT (silencing mediator of retinoid and thyroid receptors) (Jackson et al., 1997; Lavinsky et al., 1998). The co-repressors SMRT and N-CoR establish an unfavourable chromatin structure for transcriptional activation as a result of their histone deacetylase activity (Yang et al., 1996), thereby finally leading to the repression of transcription of SHRs (Labrie et al., 1999). These co-repressors may also compete with the SRC proteins, demonstrating that the balance of co-activators and co-repressors may finally regulate transcription (Torchia et al., 1998;
Beato et al., 2000). SHRs can exist in different conformational states, each of which exhibits a different degree of transcriptional activity. The degree of these ligand-induced conformational changes in the SHRs regulates the interaction of the receptor with co-activators (Wagner et al., 1995).

Co-activator mRNA for SRC-1, GRIP (glucocorticoid receptor interacting protein)-1, RAC (receptor-associated co-activator)-3 and transcriptional co-factor p300 and a co-repressor mRNA for SMRT have been shown to be expressed in human endometrial stromal cells and in the female reproductive system in rat uteri (Brosens et al., 1999; Nephew et al., 2000). However, whether or not mRNA expression levels of SHR co-factors in human endometrium might vary in response to the action of steroid hormones during the natural menstrual cycle remains to be clarified. Therefore, the objective of this study was to assess the presence of the steroid hormone receptor co-factors SRC-1, N-CoR and SMRT in human endometrium during the menstrual cycle at mRNA and protein levels using a real-time RT–PCR assay and immunohistochemistry respectively.

Materials and methods

Tissue collection

Samples of proliferative and secretory endometrium were obtained by hysterectomy or curettage specimens from premenopausal women who underwent surgery for benign disease including uterine leiomyoma or ovarian lesions and who had no history of endometriosis. The samples of menstrual phase endometrium were taken from women who attended our out-patient family planning clinic at day 3 of the menstrual cycle. In addition, we analysed inactive endometrial samples obtained by hysterectomy or curettage specimens from post-menopausal women. All premenopausal women had regular menstrual cycles, and none of the post-menopausal women had received hormonal therapy within 3 months prior to recruitment into the study. Immediately upon receipt, all endometrial samples were snap-frozen in liquid nitrogen and then stored at −80°C until further use. Specimens were dated according to the histological criteria of Noyes et al. (Noyes et al., 1975) and the patient’s testimony. We classified five phases of the menstrual cycle: the menstrual phase, the proliferative phase, and the early, mid- and late secretory phases. The collection of tissue was approved by the University of Vienna Institutional Ethical Committee.

RNA isolation and cDNA synthesis

Total RNA was extracted from deep frozen endometrial tissue by using the Tri reagent method from MRC (Molecular Research Center, Inc., OH, USA) and quantified by measuring the optical density at 260 nm. The integrity of RNA was assessed by agarose gel electrophoresis. RT was performed on 500 ng of total RNA using 200 units M-MLV Reverse Transcriptase (ViennaLab, Labordiagnostika GmbH, Vienna, Austria) and a commercially available reagent kit (Random Primed RT-Mix; ViennaLab). Synthesized cDNA was stored in aliquots at −80°C.

Real-time PCR analysis

All amplification primers and TaqMan probes used (Table I) were designed with Primer-Express software (PE Biosystems, Foster City, CA, USA). FAM (6-carboxyfluorescein) was used as the reporter dye and TAMRA (6-carboxytetramethylrhodamine) was used as the quencher dye. Oligonucleotide synthesis and purification was performed by VBC-Genomics Bioscience Research GmbH (Vienna, Austria). The reactions were carried out in 25 µl total volume containing 2 µl cDNA, 25 pmol of each amplification primer, 5 pmol probe and 12.5 µl 2×TaqMan Universal Mix (PE Biosystems). Reaction conditions were 50°C for 2 min, 95°C for 10 min (activation of the AmpliTag-Gold polymerase), followed by 40 cycles of 15 s at 95°C (denaturation) and 60 s at 60°C (annealing and extension).

In order to correct for variations linked to differences in the amount of RNA taken for the reaction, or for different levels of inhibition during RT or PCR, we normalized expression levels using the β-2-microglobulin gene, which is a ubiquitously expressed housekeeping gene, as a reference gene.

The expression of this housekeeping gene was quantified with the human β-2-microglobulin TaqMan Assay Kit from PE Biosystems according to the manufacturer’s guidelines. All experiments were carried out in triplicate and several negative controls were included. Fluorescence emission was continuously monitored and analysed by a GeneAmp 5700 Sequence Detection System (PE Biosystems) with the GeneAmp 5700 SDS Software (Version 1.1).

Standard curves

For the generation of standard curves, we used 2-fold serial dilutions of cDNA samples, which showed the highest mRNA expression levels in real-time RT–PCR, as templates. The standard curves were constructed and calculated by GeneAmp 5700 SDS Software, referring the threshold cycle (PCR cycle at which a specific fluorescence becomes detectable) to the log of the cDNA starting quantity of each dilution step. These standard curves allowed us to interpolate the unknown mRNA expression levels in each analysed sample.

Immunohistochemistry for SRC-1 and N-CoR

Frozen sections were mounted onto silane-coated slides. To quench endogenous peroxidase activity, specimens were incubated with peroxidase blocking agent (0.3% hydrogen peroxide) for 5 min. The slides were again rinsed in phosphate-buffered saline (PBS), transferred to a PBS wash for 2 min and the supernatant was discarded. The slide sections were incubated in serum block for 4 min (goat or donkey serum, dependent on the primary antibody: Immunocruz Staining Systeme, Santa Cruz Biotechnology, CA, USA). After dipping in PBS, the slides were then incubated with SRC-1 (Santa Cruz M-341, diluted 1:50 in PBS; 90 min at room temperature) or N-CoR antibodies (Santa Cruz H-303, diluted 1:20 in PBS; 90 min at room temperature). Negative controls were performed for all tissue sections replacing primary antibodies by proportionately diluted isotype immunoglobulins (Immunno Cruz Staining System). MCF-7 and T47D breast carcinoma cell lines were used as positive controls.

The slides were incubated with a mouse, rabbit or goat biotinylated secondary antibody provided in the Immunno Cruz detection kit (Santa Cruz Biotechnology) and then horse-radish peroxidase agent for 60 min. Finally, they were incubated with the substrate chromogen 3,3′-diaminobenzidine for 5 min. The sections were counterstained with haematoxiline for 20 s. All incubations were conducted at room temperature.

Immunostaining quantitation: a semi-quantitative scoring system (immunoreactive score; IRS) was used to improve the evaluation of the immunohistochemically stained sections (Remmele et al., 1993; Remmele score). The IRS (negative, weak, moderate and strong) staining intensity, which is multiplied with the percentage of positive cells or nuclei, ranges between 0 and 12 points. No staining cut-off was defined as 0–2 points, which was related to the biochemical value of 10–20 fmol/mg tumour protein. Weak staining (3–5 points) was defined as ‘+’, moderate staining (6–8 points) as ‘+’ and strong staining (9–12 points) as ‘++’ and ‘+++’. Here, for practical reasons, we combined moderate and strong staining (‘+’ and ‘++’) and summarized it as ‘+’.

Statistics

The statistical data were evaluated on a personal computer using the SPSS statistical program (‘Superior Performing Software Systems’, Chicago, USA) version 10.0. The differences between the phases of the menstrual cycle were tested by the Mann–Whitney U-test and P < 0.05 was considered significant.

Results

The endometrial specimens (n = 56) were classified as follows: menstrual phase [n = 16, corresponding to cycle day (c.d.) 1–5], proliferative phase (n = 15, corresponding to c.d. 9–12), secretory phase (n = 17, corresponding to c.d. 17–24; early secretory phase: n = 7; mid-secretory phase: n = 6; late secretory phase: n = 4) and inactive endometrium (n = 8, from post-menopausal women). Endometria used in this study all showed normal morphology.

Demonstration of SRC-1, N-CoR, and SMRT gene expression

All the tissues examined (n = 42) were found to express SRC-1, N-CoR and SMRT mRNA.

Increased SRC-1 and N-CoR expression during menstruation
Table I. Sequences and genomic position

<table>
<thead>
<tr>
<th>Name</th>
<th>Sequence (5’–3’)</th>
<th>Position (GB accession no.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SMRT – F</td>
<td>GGTCAAGTCCAGAAAGGAGAT</td>
<td>pos. 4275–4298 (U37146)</td>
</tr>
<tr>
<td>SMRT – R</td>
<td>GCTTCTAGGTCATAGGGAATCTTTG</td>
<td>pos. 4391–4416 (U37146)</td>
</tr>
<tr>
<td>SMRT – probe</td>
<td>TGAAACACGACACACGGAATGAC</td>
<td>pos. 4310–4333 (U37146)</td>
</tr>
<tr>
<td>hN-CoR – F</td>
<td>TGAAACACCATAGGATGTTAAGGCA</td>
<td>pos. 6138–6161 (AF044209)</td>
</tr>
<tr>
<td>hN-CoR – R</td>
<td>GGTAAGGATCTGTCAGCTTTGA</td>
<td>pos. 6240–6261 (AF044209)</td>
</tr>
<tr>
<td>hN-CoR – probe</td>
<td>AGACCTATCCAGACAGGTTGTTAAGGCA</td>
<td>pos. 6209–6237 (AF044209)</td>
</tr>
<tr>
<td>SRC-1 – F</td>
<td>AACCTCAGCGGCAACTTACA</td>
<td>pos. 2248–2268 (U40396)</td>
</tr>
<tr>
<td>SRC-1 – R</td>
<td>CCTCAAAAGCCAGGTCAGTTGAC</td>
<td>pos. 2303–2324 (U40396)</td>
</tr>
<tr>
<td>SRC-1 – probe</td>
<td>CAAACAGGCCGCAGGAGGCTAATAC</td>
<td>pos. 2271–2298 (U40396)</td>
</tr>
</tbody>
</table>

F = forward. R = reverse.

Figure 1. Summary plot based on the median, quartiles and extreme values. The box represents the interquartile range which contains 50% of values. The whiskers are lines that extend from the box to the highest and lowest values, excluding outliers. A bold line across the box indicates the median. Menstrual endometrium was compared with the proliferative, secretory and inactive endometrium. The outliers did not have significant influences, hence they were not shown in the boxplot. (A) Summary plot for SRC-1 mRNA-levels in human endometrium, showing a significant increase in the menstrual phase compared with proliferative (P < 0.001) and secretory (P < 0.001) phases, and inactive endometrium (P < 0.001); (* = statistically significant). (B) Summary plot for N-CoR mRNA levels in human endometrium, showing a significant increase in the menstrual phase compared with proliferative (P < 0.001) and secretory (P < 0.001) phases, and inactive endometrium (P = 0.029); (* = statistically significant). (C) Summary plot for SMRT mRNA levels in human endometrium, showing significant differences between inactive endometrium and endometria of the various phases of the menstrual cycle.
and endometria of the various phases of the menstrual cycle. mRNA levels did not differ significantly between the early, mid- and late secretory phases. Endometrial SMRT protein were found in the functional layer of the endometrium during the different phases of the cycle, and are described as follows (Table II). The staining intensity was different across the phases. However, the staining intensity was weak through the entirety of all phases of the menstrual cycle in the stromal cells. The inactive endometrium exhibited no N-CoR staining (Figure 3F).

### Discussion

The SHR co-factors are abundantly expressed in the uterus and are thus suggested to bind to SHRs, thereby leading to activation or repression of transcription in the human endometrium and rat uterus (Brosens et al., 1999; Nephew et al., 2000). In this study, the nuclear receptor co-factors SRC-1, N-CoR and SMRT mRNA and/or protein levels were studied in the human endometrium throughout the menstrual cycle. Our results demonstrate that the SHR co-factors SRC-1 and N-CoR are differentially expressed at protein and mRNA levels during the endometrial cycle, with peak levels during menstruation.

The co-activator proteins include SRC-1, which is a nuclear co-activator sharing sequence homology with TIF2/GRIP1, and AIB1/RAC3/ACTR/TRAM-1, another member of the p160 co-activator family (Kraichely et al., 2000). SRC-1 interacts with the AF-2 domains of SHRs such as estrogen receptor α and progesterone receptor (PR) in a ligand-dependent manner and enhances their hormone-dependent transcriptional activities, without altering the basal activity of the target promoter (Onate et al., 1998). A loss of the SRC-1 gene in mice is reported to result in a lack of estradiol (E2) responsiveness in the development of female reproductive organs (Xu et al., 1998). In accordance, targeted deletion of the SRC-1 gene in mice markedly reduces the decidualization reaction in response to progesterone treatment and mechanical traumatization (Xu et al., 1998).

The SHR co-factors have been shown to be influenced by sex steroids; however, regulation of co-factors differs depending on the tissue. E2 down-regulates SRC-1 expression in the rat pituitary gland and up-regulates it in breast cancer cells (Thenet et al., 1999). In contrast, SRC-1-mRNA levels have been reported to be insensitive to E2 in the hypothalamus, the pineal gland cells and the uterus of rats (Misiti et al., 1998; Nephew et al., 2000), as well as in human endometrial cancer cells (Thenet et al., 1999). In our study, SRC-1 mRNA and protein levels were shown to be up-regulated during the menstrual phase, down-regulated during the proliferative and secretory phases, and low or absent in inactive endometria. Immunohistochemistry of SRC-1 protein has been performed in breast tissue and rat hippocampus (Shim et al., 1999; Ogawa et al., 2001). We have now found SRC-1 immunostaining to be most pronounced in the epithelial layer of menstrual phase endometrium.

The human endometrium of the menstrual phase is reported to express a high proliferative activity, with a 30% increase of Ki-67 positive stromal cells during the onset of menstruation in primates (Dahmoun et al., 1999), while there is a low proliferative activity during the secretory phase (Nisolle et al., 1997) which correlates well with the temporal pattern of SRC-1 expression shown here. In contrast to the findings of Nephew et al. who demonstrated the rat uterus as being insensitive to E2 (Nephew et al., 2000), our present findings provide evidence for a temporal pattern of co-factor expression in the human endometrium. This discrepancy between the two studies might be a consequence of the different sensitivities of the methods used, since Nephew et al. previously hypothesized Northern analysis as being insensitive for the accurate monitoring of co-activator mRNA changes. Alternatively, the difference could result from a different species being used since the rat uterus has been described not to exhibit classical menstrual bleeding. Our findings thus clearly imply a possible role of SRC-1 during human endometrial remodelling.

Transcriptional activation in the endometrium by SHRs may also involve co-repressors such as N-CoR and SMRT (Jackson et al.,

### Table II. Results of immunohistochemistry

<table>
<thead>
<tr>
<th>Endometrial dating (n)</th>
<th>SRC-1 Epithelium</th>
<th>SRC-1 Stroma</th>
<th>N-CoR Epithelium</th>
<th>N-CoR Stroma</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proliferative (3)</td>
<td>(+)</td>
<td>0</td>
<td>(+)</td>
<td>0</td>
</tr>
<tr>
<td>Early secretory (3)</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Mid-secretory (2)</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Late-secretory (1)</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Menstrual (3)</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Inactive (2)</td>
<td>(+)</td>
<td>0</td>
<td>(+)</td>
<td>0</td>
</tr>
</tbody>
</table>

A semi-quantitative scoring system (immunoreactive score; IRS) was used to improve evaluation of the immunohistochemically stained sections (Remmele score). For this study, the IRS was modified by designating moderate and strong staining because the differentiation between moderate and strong staining was not significant. The mean staining intensity of phases of the menstrual cycle was calculated with staining ‘0’, as no staining; (+), between weak and no staining; ‘+’, weak, ‘++’, moderate or strong staining.

Three groups of phases of the menstrual cycle (menstrual, late proliferative, secretory phase) were tested in pairs by the Mann–Whitney U-test. We found a significant increase in SRC-1 and N-CoR mRNA in the menstrual phase compared with samples from proliferative (P < 0.001) and secretory (P < 0.001) phases, and with samples of inactive endometrium (P < 0.001 and P = 0.029 respectively) (Figure 1A,B). The SMRT mRNA expression levels did not differ between the phases of menstrual cycle (Figure 1C). The mRNA levels of SRC-1, N-CoR and SMRT did not differ between the proliferative and secretory phases. Moreover, SRC-1, N-CoR and SMRT co-factor mRNA expression levels did not differ significantly between the early, mid- and late secretory phases. Endometrial SMRT mRNA levels did not differ significantly between inactive endometria and endometria of the various phases of the menstrual cycle.

### Cellular localization of SRC-1 and N-CoR

We did not find any differences in co-factor staining between the different phases of the menstrual cycle in the basal layer of the endometrium. In contrast, different expression levels of co-factor protein were found in the functional layer of the endometrium during the different phases of the cycle, and are described as follows (Table II).

**SRC-1**

The immunolocalization of SCR-1 was shown in all phases during the menstrual cycle (Figure 2A–D). However, the staining intensity was different with respect to menstrual cycle-related changes in endometrial morphology. In particular, nuclear immunostaining was strong or moderate in menstrual phase endometrium within both epithelial and stromal cells. In contrast, immunostaining was only weak or absent in the proliferative phase endometrium or moderate in the secretory phase. However, the difference between those phases was not great and was more pronounced in the epithelium than in the stroma. There were no clear differences in staining intensity between the early (Figure 2B), mid (Figure 2C) and late secretory phase. Within the inactive endometrium, staining was weak or not detectable in epithelium and stroma (Figure 2E).

**N-CoR**

Immunolocalization of N-CoR was shown in all phases during the menstrual cycle (Figure 3, A–D). The staining intensity was different with respect to menstrual cycle-related changes in endometrial morphology. Particularly in epithelial cells, the staining intensity was moderate or strong during the menstrual phase, but only weak or absent during the secretory and proliferative phases respectively. However, the staining intensity was weak through the entirety of all phases of the menstrual cycle in the stromal cells. The inactive endometrium exhibited no N-CoR staining (Figure 3F).
Figure 2. SRC-1 immunostaining in different phases of the menstrual cycle: (A) proliferative phase; (B) early secretory phase; (C) mid-secretory phase; (D) menstrual phase; (E) inactive endometrium; (F) mid-secretory phase (negative control). Original magnification ×400 (bar = 20 µm).

These are two transcriptional corepressors that utilize the C-terminal domain for interaction with unliganded receptors and the N-terminal domain for transcriptional repression. The effect of the co-repressors N-CoR and SMRT on the function of endocrine organs has been previously described in detail. In particular, N-CoR mRNA is reported to be expressed in rat pituitary CH3 cells, hypothalamus and pineal glands; however, N-CoR mRNA expression is not altered by E2 in those organs (Misiti et al., 1998). SMRT mRNA expression has been found to be increased by E2 in the male rat pituitary, but not in the hypothalamus or pineal glands (Misiti et al., 1998). In the rat uterus, low SMRT mRNA expression levels have been detected by Northern analysis, and this expression remains unchanged after E2 administration (Nephew et al., 2000). Here, in contrast to Nephew et al., we showed high levels of endometrial N-CoR mRNA and protein levels during menstruation compared with the other phases of the menstrual cycle.

Endometrial SMRT mRNA levels did not vary during the different phases of the menstrual cycle. Endometrial N-CoR mRNA up-regulation during the menstrual phase suggests a potential function of N-CoR as a co-repressor of a SHR gene that has to be silenced, e.g. in the case of PR, when estrogen action is required to induce endometrial proliferation in the absence of progesterone. This putative silencing effect on the PR has been hypothesized from the finding that over-expression of N-CoR partially represses agonist activity of
Increased SRC-1 and N-CoR expression during menstruation

Figure 3. Immunolocalization of N-CoR was shown in all phases of the menstrual cycle (A-D) and in the inactive endometrium (E). (A) proliferative phase; (B) early secretory phase; (C) mid-secretory phase; (D) menstrual phase; (E) menstrual phase (negative control); (F) inactive endometrium. Original magnification ×400 (bar = 20 µm).

PR-bound RU 486 (Jackson et al., 1997). Alternatively, co-repressors such as N-CoR and SMRT could have physiological functions with respect to agonist action, but might play an ‘unphysiological role’ in the presence of antagonists such as RU 486 (Jackson et al., 1997). However, the physiological relevance of the observed increase of N-CoR levels during menstruation remains to be further clarified.

The endometrial breakdown that occurs during the late secretory phase and menstruation is accompanied by a dramatic change from a progesterone dominant milieu (secretory phase) to an estrogen dominant milieu (proliferative phase). These processes are now shown to be associated with changing steroid co-factor expression levels, a finding that has probable implications for endometrial remodelling during the menstrual cycle.

In conclusion, we clearly demonstrate a fluctuation of SRC-1 and N-CoR mRNA and protein levels, showing the highest levels during menstruation when compared with the other phases of the menstrual cycle. This represents a further significant clarification of the physiological processes involved in the cyclic endometrial remodelling.

Acknowledgements
This work was supported by the Medizinisch wissenschaftlicher Fonds des Bürgermeisters der Stadt Wien (Grant 1815, Grant 1527). We appreciate the
help of Barbara Widmar for the contribution to the study and are also grateful
to Jon R.Bishop for critical review of the manuscript.

References
F.Wieser


Submitted on May 9, 2001; resubmitted on December 28, 2001; accepted on April 17, 2002.