Leukaemia inhibitory factor receptor and gp130 in the human Fallopian tube and endometrium before and after mifepristone treatment and in the human preimplantation embryo

K. Wånggren1,2,4, P.G. Lalitkumar1, F. Hambiliki2,3, B. Ståbi1, K. Gemzell-Danielsson1* and A. Stavreus-Evers2*

1 Department of Woman and Child Health, Division of Obstetrics and Gynaecology, Karolinska Institutet, Karolinska University Hospital, S-171 76 Stockholm, Sweden; 2 Department for Clinical Science, Intervention and Technology, Division of Obstetrics and Gynecology, Karolinska Institutet, Stockholm, Sweden; 3 Center for Reproduction, Department of Gynaecology, Uppsala University Hospital, Uppsala, Sweden
4 Correspondence address. E-mail: kjell.wanggren@karolinska.se

Leukaemia inhibitory factor (LIF) is a cytokine, which is associated with reproductive processes such as embryo development and implantation. The objectives of this study were to detect the presence of LIF receptor (LIFR) and glycoprotein 130 (gp 130) in the human Fallopian tube, endometrium and preimplantation embryo and to study the effect of mifepristone on the expression of LIFR and gp130 in the Fallopian tube. Twenty-two healthy fertile women received a single dose of 200 mg mifepristone or placebo immediately after ovulation (LH1). Biopsies were obtained from the Fallopian tubes during laparoscopic sterilization once between days LH1 and LH2, and from endometrium once between days LH1 + 6 and LH1 + 8. Preimplantation embryos were received from couples undergoing in vitro fertilization treatment. Immunohistochemistry was used to detect the presence of LIFR and gp130 in the Fallopian tube, endometrium and preimplantation embryo. Real-time PCR was used to study LIFR and gp130 expression in the Fallopian tube, preimplantation embryo and endometrium. LIFR was more abundant than in the endometrium. In the blastocyst, the staining of gp130 was mainly localized in the inner cell mass, whereas LIFR was expressed in all cells. The presence of LIFR and gp130 in the Fallopian tube and preimplantation embryo indicates a role for LIF in communication between the embryo and the Fallopian tube. Mifepristone did not affect the expression of LIFR and gp130 in the Fallopian tube, nor in the endometrium suggesting that progesterone might not be directly involved in the regulation of LIFR or gp130.

Keywords: embryo/endometrium/Fallopian tube/gp130/LIF receptor

Introduction

Normal microenvironment and function of the Fallopian tube are of vital importance for human reproduction. The densely ciliated fimbrial apparatus of the Fallopian tube picks up the ovum directly after ovulation. The ovum is thereafter transported into the ampulla, where it is fertilized (Pauerstein and Eddy, 1979). Following this, the embryo undergoes cleavage and development while being transported through the Fallopian tube, over a period of 3 days, after which it reaches the uterine cavity where it will hatch and implant (Croxatto et al., 1978).

Leukaemia Inhibitory Factor (LIF) is a cytokine, which regulates differentiation, proliferation and survival of various cells in the in the embryo as well as in the adult (Gearing, 1993), such as embryonic stem cells (Smith et al., 1992), primordial germ cells (Matsui et al., 1991) and peripheral neurons (Yamamori, 1992). LIF has been shown to be involved in a number of processes in reproduction such as enhancement of sperm motility and survival (Attar et al., 2003) and in the physiology of ovulation (Arici et al., 1997). LIF enhances blastocyst formation and hatching (Larvanos et al., 1995) and has been shown to be essential for implantation in mouse (Stewart et al., 1992). LIF also increases implantation rates in sheep (Fry et al., 1992). It is known that addition of LIF to the culture medium significantly increases blastocyst formation in human embryos (Dunglison et al., 1996).

The importance of LIF in human implantation is still uncertain. Infertile women show dysfunction in LIF production and it has been observed that infertile women have less LIF in uterine secretion than women with proven fertility (Laird et al., 1997; Hambartsoumian, 1998; Lass et al., 2001). However, the LIF content in uterine secretion at the time of oocyte retrieval does not seem to be correlated to pregnancy rate (Olivennes et al., 2003).

LIF exerts its biological effects by interaction through its specific LIF receptor (LIFR). The LIFR forms a high affinity heterodimer complex with glycoprotein 130 (gp130) (Robinson et al., 1994). The gp130 trans-membrane subunit can also, as a monomer, act as
interleukin-6 (IL-6), interleukin-11 (IL-11) and ciliary neutrophil factor (CNTF) receptor (Auernhammer and Melmed, 2000).

Expression of mRNA activity for LIFR and gp130 has been demonstrated in human preimplantation embryos (Sharkey et al., 1995; Chen et al., 1999), but the localization of LIFR and gp130 in human preimplantation embryos has to our knowledge not been reported.

LIF is produced in the human Fallopian tube. The highest levels are seen in the luminal epithelium of the ampulla, where the embryo first develops before its transport into the uterine cavity for implantation (Keltz et al., 1996; Li et al., 2004). Co-culture of mouse embryos with human oviductal cells will result in maintained mitochondrial function, decreased apoptosis in the embryo and a higher degree of blastocyst formation and hatching (Xu et al., 2000, 2001, 2003). Cultured bovine oviductal cells synthesize LIF that conditions the embryo for implantation (Reinhart et al., 1998). LIFR and gp130 are also of great importance at later stages of embryogenesis and fetal development (Lavranos et al., 1995; Modric et al., 2000, Mitchell et al., 2002).

The expression of LIF and its receptor in human endometrium increases around the time of implantation and was demonstrated to appear simultaneously with pinopodes (Aghajanova et al., 2003). LIF is believed to be a regulatory factor essential for implantation of the blastocyst through autocrine and paracrine interaction between LIF and its receptor in the luminal epithelium (Cullinan et al., 1996; Cheng et al., 2002). Gp130 is most abundantly expressed at the time of implantation (Classen-Linke et al., 2004), when increased secretion of soluble gp130 was seen (Sherwin et al., 2002). LIFR and gp130 mRNA is also expressed in the decidua and may play a key role during the decidualization and placentation (Ni et al., 2002). It has been shown that the secretion of gp130 was reduced in infertile women (Sherwin et al., 2002).

Progesterone is essential for the development of endometrial receptivity. Treatment with a progesterone receptor antagonist, such as mifepristone, directly after ovulation reduces endometrial LIF expression at the expected time of implantation (Cameron et al., 1997; Daniellsson et al., 1997). Mifepristone acts by competitive binding to the progesterone receptor. (Rauch et al., 1985; Teutsch et al., 1988). Treatment with mifepristone increases the expression of progesterone receptors (Christow et al., 2002; Sun et al., 2003) but has no effect on the expression LIF in the human Fallopian tube (Li et al., 2004).

Taken together LIF is an important cytokine in the female reproductive tract and in the preimplantation embryo. Still data on LIF and LIF receptor regulation is scarce and very little is known about possible communication between the embryo and the Fallopian tube. Therefore, the objective of the present study was to reveal the localization of LIFR and gp130 in the human Fallopian tube and preimplantation embryo and, in addition, to study the effect of mifepristone on these receptors in the human Fallopian tube and endometrium.

Materials and methods

Study subjects

Twenty-two healthy women (age 31–44 years) were included in the study. All women had proven fertility and had regular menstrual cycles (range 25–35 days). None of the women had taken any hormonal treatment or used an IUD for at least 3 months prior the study.

Collection of Fallopian tube tissue

Sixteen women were randomly allocated to receive treatment with a single dose of 200 mg mifepristone (n = 8) immediately after ovulation (LH+2) or to a control group (n = 8). Randomization was performed using opaque, numbered and sealed, envelopes. Laparoscopic sterilization was performed by placement of a silicone rubber ring over a segment of the isthmic part of the Fallopian tube (Yoon and King, 1975). The surgery was performed on day LH+4 to LH+6, which corresponds to the time of development and transport of the preembryo within the Fallopian tube. At surgery, biopsies were obtained from both Fallopian tubes. On one side biopsies were obtained from the isthmic part and on the other side from the ampullary part. The biopsies were immediately snap-frozen and stored in liquid nitrogen until analysed.

Collection of endometrial tissue

Endometrial biopsies were obtained from six healthy women during a control cycle on day LH+6 to LH+8, which corresponds to the assumed time of embryo implantation (Martel and Psychoyos, 1981; Nikas et al., 1995). An additional biopsy was obtained from the same women in the following cycle, after treatment with a single dose of 200 mg mifepristone immediately after ovulation (LH+2). The biopsies were obtained by curettage of the uterus using a Randall curette without prior dilatation of the cervix. Samples for immunohistochemistry were fixed in 4% formaldehyde for a maximum of 24 h and then stored in 70% ethanol until embedding. Samples for real-time PCR were snap-frozen and stored in liquid nitrogen until analysed.

Collection of human preimplantation embryos

Eighty human preimplantation embryos were received from patients undergoing in vitro fertilization (IVF) treatment. The preimplantation embryos were in all different stages from 2 cell embryos to hatching blastocysts.

Ethics

The study was approved by the local ethics committee at the Karolinska University Hospital/Karolinska Institutet. All women gave their written informed consent before entering the study. The embryos used in this study were donated by couples undergoing IVF treatment. Only embryos that could not be used for infertility treatment were used in the present study. Both partners in the couple signed an informed consent form after receiving oral and written information. No reimbursements were given to the couples.

Menstrual cycle monitoring

The day of the LH surge was estimated using a self-test detecting urinary LH (Clearplan, Searle Unipath, Bedford, UK) twice daily from cycle day 10 to the LH peak. In addition, all women collected daily urine during the cycle for analysis of estrone- and pregnanediol-glucuronide and LH using radioimmunoassay (Cekan et al., 1986).

Immunohistochemistry of Fallopian tube and endometrium

Biopsies from the Fallopian tube were mounted in an embedding medium (OCT Compound; Miles Inc., Elkhart, IN, USA) and serially sectioned to 9 µm using a Reichert-Jung Cryocut 1800 (Cambridge Instruments GmbH, Nussloch). The sections were mounted on glass slides and immersed in 2% parafomaldehyde in phosphate-buffered saline (PBS). The mounted sections were then wrapped in parafilm and stored in −70°C until use.

Paraffin-embedded biopsies from the endometrium was sectioned to 4 µm and mounted on glass slides. The samples were thereafter deparaffinated in BioClear (CIAB, Stockholm, Sweden) and rehydrated in decreasing concentrations of ethanol ending up in PBS.

All samples from the Fallopian tube and endometrium were hereafter treated in the same way. The samples were rinsed in PBS, incubated in darkness for 30 min in H2O2 (0.3 % in methanol) to block endogenous peroxidase activity and washed with PBS/BSA (Albumin, Bovine 0.05%). Hereafter the slides were blocked with 10% horse serum (in PBS/BSA) for 30 min. The sections were then incubated with the primary antibody, diluted 1:50 for LIFR and 1:25 for gp130, over night at 4°C. The primary antibody for LIFR was an affinity-purified goat polyclonal antibody (AF-249-NA, R&D Systems Inc.), raised against human LIFR. The antibody for gp130 was a goat polyclonal antibody (AF-228-NA, R&D Systems Inc.), raised against gp130 of human origin. For negative control, the primary antibody was replaced with non-immune serum of equivalent concentration from the same species. The slides were washed in PBS/BSA and thereafter incubated with the secondary antibody diluted 1:300 (horse anti-goat) for 30 min at room temperature. The slides...
were then rinsed in PBS/BSA, prior to incubation with ABC complex (Vectastain Elite ABC immunoperoxidase detection system, Vector Laboratories Inc., Burlingame, CA, USA) according to the manufacturer's instructions. After washing with PBS/BSA, freshly prepared diaminobenzidine-hydrogen peroxide solution (DAB kit from Vector) was added to the slides, which were thereafter rinsed with distilled water. The slides were counterstained with 10% Mayer's Haematoxylin (VWR, Stockholm, Sweden), then washed in cold water and mounted with glycerol-gelatine.

Two persons evaluated the immunohistochemical staining independently, blinded to the identity of the samples. When the evaluation of the slides turned out differently, the average value was used. The staining was graded on a scale of 0 = no staining of cells, + = faint staining, +/− = moderate staining and +/+ = strong staining.

**Immunohistochemistry of embryos**

The embryos were rapidly transferred from culture and briefly washed in PBS containing 3 mg/ml polyvinylpyrrolidone (PVP) and then fixed with 2.5% paraformaldehyde in PBS for 15 min at room temperature. Following fixation, the embryos were permeabilized in PBS/PVP buffer containing 0.25% Triton X 100 for 30 min. Thereafter the embryos were placed in PBS blocking buffer containing 0.1% BSA and 0.01% Tween 20 for 15 min. The same primary antibodies as used for the endometrium and Fallopian tube were added in blocking buffer. For negative control, the primary antibody was replaced with non-immune serum of equivalent concentration from the same species. The embryos were then washed three times for 15 min each in blocking buffer to remove any unbound primary antibodies. The secondary antibodies, Alexa 568 conjugated rabbit anti-goat (1:250), were diluted in blocking buffer and applied to the embryos for 60 min at RT in the dark. After incubation, the embryos were briefly washed through a series of 25, 50, 75 and 100% citifluor (with DAPI) and then mounted on slides in antifade medium under a cover slip. Stained embryos were viewed in an inverted microscope (Zeiss Axiovert 200M, Germany) equipped with fluorescence optics and appropriate filters.

**RNA and cDNA preparation**

Total RNA was isolated using SV Total RNA Isolation System (Promega Corp., Madison, WI, USA) according to the manufacturer’s protocol. One microgram of each of the total RNA from each sample was reverse transcribed using deoxynucleotide triphosphates (10 mM each), random hexamer (250 ng/µg) and Superscript reverse transcriptase (200 U/µl), using the Superscript™ II RNase H− Reverse Transcriptase Kit (Invitrogen, Stockholm, Sweden).

**Real-time PCR**

Taqman real-time PCR (Applied Biosystems, Foster City, California, USA) was used to quantify the differential expression of LIFR and gp130 in the Fallopian tube (n = 9) and endometrium (n = 4).

Experiments were performed on a 96 well array format using the ABI PRISM 7300 instrument (Applied Biosystems) incorporating the target assay genes for LIFR or gp130 along with eukaryotic 18S rRNA as an internal control, which was used to normalize the expression levels of target genes in each sample. cDNA form human brain (Ambion, Austin, TX, USA) was used as positive control for the expression of both LIFR and gp130. Fluorescent probes were 5′ labelled with 6-carboxyfluorescein (FAM) and 3′ labelled with MGB non-fluorescent quencher. Fluorescent signal detection used ‘ROX’ as the internal passive reference dye. The inouculm (25 µl) consists of 12.5 µl Taqman universal PCR master mix (Applied Biosystems), 1.25 µl assay probe (LIFR: Assay ID Hs00158730_m1, assay location bas 137 in the reference sequence NM_002310.3, exons 1–2. The length of the product is 63. GP130: Assay ID Hs00174360_m1, assay localization base 2016 in the reference sequence NM_175767.1, exons 13–14 and assay location base 2099 exons 14–15. The length of the product is 72, Applied Biosystems), 6.25 µl RNase/DNase free sterile water and 5 µl of diluted (1:2.5) cDNA. A standard curve was performed for both GP130 and LIFR using serial dilution of cDNA synthesized from endometrium, Fallopian tube and brain. After identifying the appropriate dilution for the respective samples, the assay was performed as follows: initial denaturation was carried out for 10 min at 95°C, followed by 40 cycles of denaturation at 95°C for 15 s and combined primer annealing/ extension at 60°C for 1 min. All reactions were performed in triplicate and the mean value of the threshold cycle (Ct, the start of exponential amplification) of each sample was normalized with their respective threshold cycle of 18S, obtaining the ΔCt value. Data was analysed using SDS 1.2.3 software (Applied Biosystems). Relative expression was calculated using the following formula 100 × 2−ΔDCT as is described in User Bulletin number 2 from Applied Biosystems.

**Statistics**

Nonparametric statistical evaluation, ANOVA on ranks, was performed for differences in staining intensity for LIFR and gp130. Mann–Whitney Rank Sum Test was performed to compare the mRNA levels. A P-value of <0.05 was considered statistically significant.

**Results**

**LIFR in the Fallopian tube**

The most intense immunostaining was seen in the apical and basolateral side of the luminal epithelial cells (Fig. 1A), in vessels (Fig. 1B) and in the serosal epithelium of the Fallopian tube (Fig. 1C). There was no difference in staining intensity between the ampullary and the isthmic part of the Fallopian tube. Treatment with mifepristone did not affect the staining intensity in any compartment of the Fallopian tube (Figs. 1E–1G and 2A).

**Gp130 in the Fallopian tube**

Faint immunostaining for gp130 was seen in the luminal epithelium of the Fallopian tube predominately in the isthmic part (Fig. 1I). Similar immunostaining was also seen in the muscular wall, in some vessels (Fig. 1J) and in the serosal surface epithelium (Fig. 1K). Treatment with mifepristone did not affect the staining intensity in any compartment of the Fallopian tube (Figs. 1M–O and 2B).

**LIFR in the endometrium**

Moderate immunostaining for LIFR was seen in the luminal and glandular epithelium of the endometrium (Fig. 1Q and R). Some faint staining was also seen in the endometrial stroma and in some vessels (Fig. 1Q) and in the serosal surface epithelium (Fig. 1K). Treatment with mifepristone did not affect the staining intensity in any compartment of the Fallopian tube (Figs. 1Q and S). There was no change in staining intensity after mifepristone treatment (Fig. 1S and T).

**Gp130 in the endometrium**

Moderate immunostaining for gp130 was seen in the luminal and glandular epithelium of the endometrium (Fig. 1V and W). There was no change in staining intensity after mifepristone treatment (Fig. 1X and Y).

**Comparison of LIFR in Fallopian tube and endometrium**

Taqman real-time PCR confirmed the presence of LIFR mRNA in the Fallopian tube (Fig. 3A). The relative mRNA expression of LIFR in the Fallopian tube was significantly higher than that of the endometrium, P = 0.006 (Fig. 3A).

**Comparison of gp130 in the Fallopian tube and endometrium**

Real-time PCR confirmed the presence of gp130 mRNA in the Fallopian tube (Fig. 3B). There was no significant difference in the relative expression between the mRNA levels of gp130 in the Fallopian tube compared with the endometrium, P = 0.792 (Fig. 3B).
Figure 1: Representative pictures of immunostaining for LIFR and gp130 in the Fallopian tube (A–P) and endometrium (Q–Z) with and without mifepristone treatment are shown. (Arrows indicating staining in: LE, luminal epithelium; V, vessels; S, serosal epithelium; GE, glandular epithelium). (A) LIFR in luminal epithelium without treatment. (B) LIFR in muscular layer without treatment. (C) LIFR in serosal epithelium without treatment. (D) Negative control. (E) LIFR in luminal epithelium after mifepristone treatment. (F) LIFR in muscular layer after mifepristone treatment. (G) LIFR in serosal epithelium after mifepristone treatment. (H) Negative control. (I) gp130 in luminal epithelium without treatment. (J) gp130 in muscular layer without treatment. (K) gp130 in serosal epithelium without treatment. (L) Negative control. (M) gp130 in luminal epithelium after mifepristone treatment. (N) gp130 muscular layer after mifepristone treatment. (O) gp130 in serosal epithelium after mifepristone treatment. (P) Negative control. (Q) LIFR in luminal epithelium without treatment. (R) LIFR in glandular epithelium without treatment. (S) LIFR in luminal epithelium after mifepristone treatment. (T) LIFR in glandular epithelium after mifepristone treatment. (U) Negative control. (V) gp130 in luminal epithelium without treatment. (W) gp130 in glandular epithelium without treatment. (X) gp130 in luminal epithelium after mifepristone treatment. (Y) gp130 in glandular epithelium after mifepristone treatment. (Z) Negative control.
LIFR in human preimplantation embryos
Fluorescence microscopy showed presence of LIFR in all preembryonic stages and in all cell types from the 2 cell stage to the expanded blastocysts (Fig. 4A–F).

Gp130 in human preimplantation embryos
Fluorescence microscopy showed staining of gp130 in the embryonic cells. Embryos up to the morula stage showed staining in all cells (Fig. 4H–K). In early blastocysts and in a hatching blastocyst, the gp130 staining was located to the inner cell mass (Fig. 4L and M).

Discussion
It is known that LIF is present in the human embryo (Chen et al., 1999; Sharkey et al., 1995) endometrium and Fallopian tube (Senturk and Arici, 1998). The present study shows the presence of LIFR and gp130 in the luminal epithelium of the Fallopian tube and in preimplantation embryos, suggesting paracrine interactions involving LIF between the developing embryo and the Fallopian tube in addition to autocrine interactions occurring within the developing embryo and the Fallopian tube.

Under the influence of progesterone there is a ‘cross-talk’ or exchange of signals between the developing embryo and the maternal tract to ensure synchronization between the blastocyst and the receptive endometrium and thus render implantation possible (Hombach-Klonisch et al., 2005). Progesterone is a key hormone in human embryo implantation (De Ziegler et al., 1994; Jabbour et al., 2006). In the endometrium, the highest levels of LIF and LIFR are seen during the midsecretory phase, when serum progesterone levels are high (Charnock-Jones et al., 1994; Kojima et al., 1994; Arici et al., 1995; Aghajanova et al., 2003).

Mifepristone is a potent antiprogestin that blocks the action of progesterone at the receptor level (Klein-Hitpass et al., 1991; Gemzell-Danielsson et al., 1993). Treatment with mifepristone has earlier been shown to increase the progesterone receptor level in the Fallopian tube (Sun et al., 2003). Administration of 200 mg mifepristone on day LH+2 has been shown to be a highly effective contraceptive method (Gemzell-Danielsson et al., 1993) probably acting mainly by adversely affecting endometrial receptivity (Gemzell-Danielsson and Hamberg, 1994). Although this regimen had no effect on LIF expression in the Fallopian tube on day LH+4 to +6, the same treatment resulted in significantly reduced expression of LIF in endometrial glandular epithelium on day LH+6 to +8 (Li et al., 2004). A daily low dose contraceptive regimen of mifepristone had similar
effect on endometrial LIF expression at the expected time of receptivity (Cameron et al., 1997). The importance of LIF in endometrial receptivity and implantation is further supported by studies of cytokines in hydrosalpingeal fluid that show the presence of LIF in 50% of patients with hydrosalpinges (Strandell et al., 2004). Removal of hydrosalpinges in infertile patients resulted in increased endometrial LIF expression at the time of implantation (Seli et al., 2005) and improved results after IVF treatment (Strandell et al., 2001). Furthermore, in infertile women uterine flushings at the time of implantation demonstrated significantly lower amounts of LIF compared with fertile women (Laird et al., 1997). It has also been shown that the secretion of gp130 was lower in infertile women compared with fertile women (Sherwin et al., 2002).

In the present study, higher levels of LIFR mRNA were found in the Fallopian tube compared with the endometrium. The human embryo is known to produce LIF (Chen et al., 1999). The presence of LIFR in the Fallopian tube emphasises the importance of LIF for the tubal microenvironment. The present study shows that gp130 is located to the inner cell mass in the hatching blastocyst, whereas LIFR did not seem to be localized to a specific cell type. Therefore, it could be suggested that the targets of LIF produced by the blastocyst is the cells in the inner cell mass or the endometrium rather than autocrine/paracrine interactions with the cells in the trophoblast. The mechanism in the Fallopian tube might be different, where LIF produced by the embryo acts both within the embryo and with the Fallopian tube in an auto- and paracrine manner. This might explain why there are higher levels of LIF in the Fallopian tube than in the endometrium. In contrast, gp130 expression showed no difference between The Fallopian tube and endometrium. This may be due to the fact that gp130 alone binds other cytokines such as IL6, which is known to be produced by the human embryo (Austgulen et al., 1995). LIFR and gp130 did not change after mifepristone treatment. This indicates that progesterone is not the main regulator of LIFR and gp130 in the Fallopian tube and endometrium. In immortalized endometrial epithelial cells, LIF, LIFR and gp130 were unaffected after oestrogen and/or progesterone treatment (Hombach-Klonisch et al., 2005). This was also seen in the western spotted skunk, where prolactin, rather than progesterone, had an effect on the LIFR expression (Passavant et al., 2000). However, in endometrial epithelial and in endometrial decidual cells, a significant increase in secretion of soluble gp130 was observed after estradiol/progesterone treatment (Classen-Linke et al., 2004).

In conclusion, we found expression of LIFR and gp130 in the human Fallopian tube where it may facilitate the paracrine interaction with the embryo during its transport into the uterus. Furthermore, the staining pattern of LIFR and gp130 in the human preimplantation embryo suggests an embryonic regulation of the communication through LIFR and gp130. The communication through LIFR between the preembryo and the Fallopian tube might be important for the embryo development, and/or establishment of pregnancy. In addition, we conclude that progesterone is not likely to be a direct regulator of these receptors.

Acknowledgements

Authors thank the research nurses Lena Ellfors-Söderlund and Margareta Hellborg and the staff at the gynaecological wards at the Karolinska University Hospital, Stockholm, Sweden, for excellent care of the volunteers and patients. Also special thanks to Associate Prof. Sten Cekan and Anna Hildenbrand for valuable assistance with the manuscript. The study was supported by grants from the Center for Health Care Sciences, the Swedish Research Council (2003-6392, 2005-7293), ALF, The Swedish Society of Medicine, Karolinska Institutet and the Ake Wibergs Foundation.

References


