Endometrial protein secretion with respect to endometrial and ovarian function

Markku Seppälä, Anne-Maria Suikkari, Riitta Koistinen, Leena Riittinen and Mervi Julkunen

Department I of Obstetrics and Gynaecology, Helsinki University Central Hospital, Helsinki, Finland

Endometrial protein; Ovary

Introduction

Morphological changes take place in the endometrium in response to endocrine stimuli during the menstrual cycle, pregnancy and the menopause. The major changes include proliferation under the influence of oestrogens, followed by secretory and decidualised changes under the action of oestrogens plus progesterone. Atrophy follows sustained absence of oestrogen.

The endometrium synthesizes and secretes a number of proteins. These include 17β-hydroxysteroid dehydrogenase, IGF-binding protein (IGFBP-1), endometrial protein 14, α_1-pregnancy-associated endometrial globulin (α_1-PEG), placental protein 12 (PP12), endometrial protein 15, placental protein 14 (PP14), α_2-pregnancy-associated endometrial globulin (α_2-PEG), pregnancy-associated plasma protein-A, placental protein 5, just to name a few. For review see Seppälä et al. 1988 [1].

While ovarian steroid hormones have their well-known effects on endometrial morphology, their effects on endometrial protein secretion have been studied only recently. No feed-back mechanisms have been reported to exist from the endometrium to the ovary. We review here our studies on two proteins, namely IGFBP-1 and PP14, which both are synthesized and secreted by secretory endometrium [2,3].

Insulin-like growth factor binding protein (IGFBP-1)

Background

Previous studies have shown that placental protein 12 (PP12) is synthesized by secretory endometrium under the influence of progesterone which enhances PP12 release in endometrial tissue culture [2]. PP12 and α_1-PEG are immunologically similar [4]. The first association with an endometrial protein with IGF-1 was reported for PP12. Analysis of its N-terminal amino acid sequence disclosed identity with amniotic fluid somatomedin-binding protein, and PP12 was found to bind IGF-I (5). Later the same observation was reported on α_1-PEG [6]. Now five groups

Correspondence: M. Seppälä, Department I of Obstetrics and Gynaecology, Helsinki University Central Hospital, SF-00290 Helsinki 29, Finland.

have independently of each other cloned and sequenced the cDNA encoding this protein [7–11], designated as IGFBP-1 at the Vancouver meeting on IGF-binding proteins [12]. All but one group [7] have reported essentially the same cDNA sequence. The molecular mass of IGFBP-1 deduced from the cDNA sequence is 25.3 kDa. Southern blot analysis of genomic DNA suggests that there is a single IGFBP-1 gene within the human genome [9], and in situ hybridization studies with a cDNA encompassing the entire protein coding region of IGFBP-1 have localized the gene to bands p12-p13 on chromosome 7 [13].

Local interactions

IGFBP-1 binds IGF-1 with high affinity, similar to that of the IGF receptor [14], and IGFBP-1 has been found to compete for IGF-I with the receptor [15]. Besides IGFBP-1, IGF-1 and type I IGF receptor have also been found in the endometrium [15]. It can be postulated that IGFBP-1, induced in the endometrium by postovulatory progesterone secretion, plays a part in the transition of proliferative endometrium into the secretory phase by reducing bioavailable growth factor (IGF-I) from its receptor on the endometrial cells.

Cellular localization

Immunohistochemical studies have demonstrated this protein mainly in secretory endometrial stromal cells [16]. IGFBP-1 appears in the endometrium on day 3 or 4 postovulation, i.e., before implantation takes place in a fertile cycle [17]. IGFBP-1 mRNA has been found in secretory endometrium [9] which is capable of incorporating labelled methionine into immunoreactive IGFBP-1 in tissue culture [2]. During pregnancy, the most abundant tissue containing IGFBP-1 is decidua, and amniotic fluid is the most abundant source for purification [14].

Measurement of IGFBP-1 in serum and other biological fluids

Radioimmunoassay [18] and immunofluorometric assay [19] have been developed to estimate the levels in tissue, culture fluids and biological fluids. These assays measure the protein irrespective of whether it is bound to IGF-I or not. The levels are elevated during pregnancy [18]. There is diurnal variation in serum IGFBP-1 levels during pregnancy [20], and also in the nonpregnant state [21]. This appears to be due to meals rather than to the hour of day [22]. The levels are inversely correlated with serum insulin concentrations [23] and independent of the glucose level [24].

Apart from the endometrium, IGFBP-1 mRNA has been found in the liver [9]. Ovarian granulosa cells also synthesize and secrete this protein [25]. It has been difficult to estimate the relative contribution of each of these tissues to serum IGFBP-1 levels. The uterus appears not to be the major site in this respect, because circulating levels show no cyclical variation during the normal menstrual cycle, and hysterectomy does not bring about any sustained reduction in the levels [26]. Diurnal variation and the effect of insulin on the circulating IGFBP-1 levels are likely to reflect changes in liver-derived IGFBP-1 secretion, because insulin and IGF-I decrease the release of IGFBP-1 from Hep G2 liver cancer cells [27]. In cycles hyperstimulated for in vitro fertilization the IGFBP-1 levels rise as multiple follicles

mature [28]. This probably results from a greater granulosa cell-derived contribution to the circulating IGFBP-1 pool, because no simultaneous change takes place in the insulin levels [29].

Circulating levels in disease

In polycystic ovarian disease (PCOD) the circulating IGFBP-1 level is subnormal in one third of the patients [30]. These women are characterized by obesity, higher serum insulin and free testosterone levels and lower levels of sex hormone-binding protein (SHBG). Diet-induced weight loss is accompanied by a significant decrease in circulating levels of insulin, IGF-I and free testosterone, while the levels of SHBG and IGFBP-1 simultaneously rise [31]. Obese women with PCOD may have hyperinsulinaemia and insulin resistance, and high insulin and IGF-I levels are thought to increase ovarian androgen production [32].

Weight loss can bring about considerable metabolic changes in the endocrine control of ovarian function by decreasing insulin and IGF-I secretion [31]. The decreasing effects of insulin and IGF-I on IGFBP-1 and SHBG secretion probably take place in the liver, as both insulin and IGF-I decrease the release of IGFBP-1 and SHBG from HepG2 liver cancer cells [27]. Patients with insulinoma have subnormal IGFBP-1 levels which normalize after removal of the tumour [23].

During pregnancy the serum IGFBP-1 levels are elevated above the normal distribution in pre-eclampsia [33] and in fetal growth retardation [34]. Elevated levels have also been observed in nonpregnant women with ovarian cancer [35] and liver cancer [36]. Luteinized granulosa cells [37] as well as granulosa cell tumours contain IGFBP-1.

Placental protein 14

Background

This protein was first isolated from the human placenta [38]. Later studies have shown that PP14 is not placenta-derived. It is synthesized by secretory/decidualized endometrium which tissues contain specific PP14 mRNA [39] and are capable of incorporating labelled methionine into immunoreactive PP14 [3]. PP14 is immunologically indistinguishable from progestagen-associated endometrial protein (PEP) [40] which, in turn, is immunologically related to α-uterine protein (AUP) [41]. PP14 is also immunologically similar to α_2-PEG [4] and chorionic α_2-microglobulin [42],

Structural analyses

The first studies on the N-terminal amino acid sequence of PP14 revealed significant homology between PP14 and β-lactoglobulins of various species, most notably horse β-lactoglobulin [43]. Subsequently similar homology was reported for α_2-PEG [44]. Homology has also been observed between PP14 and a group of carrier proteins, e.g., human retinol binding protein [45]. Cloning and sequencing of the entire cDNA of PP14 has confirmed and expanded these homologies [39].

Localization in tissue

PP14 is localized to endometrial secretory glands, not to the stroma [3,46]. Also deep basal glands in the immediate postmenstrual period may occasionally contain

progesterone [56]. The role of non-steroidal compounds in the maturation of endometrial secretory capacity is currently under extensive investigation.

Patients with endometriosis exhibit similar cyclical variation in their serum PP14 levels as do apparently healthy ovulatory women. When only the low midcycle levels are compared, those women with advanced endometriosis have higher levels than those with a local disease [57]. Radical surgery brings the levels down as well as sustained treatment with danazol or medoxyprogesterone acetate [57]. In patients with endometriosis, no difference has been found in serum PP14 levels between those samples which have an elevated progesterone level and those in which the level is low. Thus factors other than progesterone may also have an influence on the serum PP14 level. Overlap between PP14 levels in normal women and those with endometriosis is considerable and invalidates the use of serum PP14 measurement as a guide to detect residual disease. This is probably due to coexisting PP14 secretion by the normal endometrium.

Our studies have shown that PP14 can be induced in the endometrium of postmenopausal women by oestrogen-progestogen replacement treatment. In postmenopausal women the serum PP14 level is low before treatment and, in a replacement cycle, it becomes elevated during the last week of cyclical progestogen [58]. The mean increase is 48% from the starting concentration. A significantly smaller increase (7%) has been observed in hysterectomized women [45]. The origin of this slight increase is not known. These studies indicate that the major part of circulating PP14 is uterus-derived. We have also found remarkable differences in the progestogen-PP14 responses between different postmenopausal women. Some exhibit a more than 100% rise, whereas others exhibit no rise at all. It is not known whether similar differences would exist in normally ovulating women with unexplained infertility. Potentially the serum PP14 measurement should be useful in detection of primary endometrial unresponsiveness, as indicated by the variable responses to hormone replacement therapy [58].

The role of PP14 in the regulation of fertility has not been established. PP14 may have immunosuppressive properties [59]. This would indicate an important role for implantation of the blastocyst. Joshi and his coworkers have reported higher serum PEP levels in the luteal phase of fertile than infertile cycles [60]. If this is confirmed by other studies, then it would be plausible to identify those women whose serum PP14 levels are not adequately elevated in the luteal phase and to treat them with additional oestrogen and progesterone.

Acknowledgements

This study is supported by grants from The Finnish Social Insurance Institution, The Academy of Finland, and the Sigrid Juselius Foundation.

References

1 Seppälä M, Huhtala M-L, Kauppila A, Rönnberg L, Vartiainen E, Julkunen M. Uterine protein secretion with respect to endocrine stimuli. Adv Gynecol Endocrinol 1988;1:417–420.

2 Rutanen E-M, Koistinen R, Sjöberg J, Julkunen M, Wahlström T, Bohn H, Seppälä M. Synthesis of placental protein 12 by human endometrium. Endocrinology 1986;118:1067–1071.

3 Julkunen M, Koistinen R, Sjöberg J, Rutanen E-M, Wahlström T, Seppälä M. Secretory endometrium synthesizes placental protein 14. Endocrinology 1986;118:1782–1786.
4 Bell SC, Bohn H. Immunochemical and biochemical relationship between human pregnancy-associated secreted endometrial α_1- and α_2-globulins (α_1- and α_2-PEG) and the soluble placental proteins 12 and 14 (PP12 and PP14). Placenta 1986;7:283–294.
5 Koistinen R, Kalkkinen N, Huhtala M-L, Seppälä M, Bohn H, Rutanen E-M. Placental protein 12 is a decidual protein that binds somatomedin and has an identical N-terminal amino acid sequence with somatomedin-binding protein from human amniotic fluid. Endocrinology 1986;118:1375–1378.
6 Bell SC, Keyte JW. N-terminal amino acid sequence of human endometrial insulin-like growth factor binding protein – evidence for two forms of the small molecular weight IGF binding protein. Endocrinology 1988;123:1202–1204.
7 Brewer MT, Stetler GL, Squires CH, Thompson RC, Busby W, Clemmons DR. Cloning, characterization, and expression of a human insulin-like growth factor binding protein. Biochem Biophys Res Commun 1988;152:1289–1297.
8 Brinkman A, Groffen C, Kortleve DJ, Geurts van Kessel A, Drop SLS. Isolation and characterization of a cDNA encoding the low molecular weight insulin-like growth factor binding protein (IBP-1). EMBO J 1988;7:2417–2423.
9 Julkunen M, Koistinen R, Aalto-Setälä K, Seppälä M, Jänne OA, Kontula K. Primary structure of human insulin-like growth factor-binding protein/placental protein 12 and tissue-specific expression of its mRNA. FEBS Lett 1988;236:295–302.
10 Lee YL, Hintz RL, James PM, Lee PDK, Shively JE, Powell DR. Insulin-like growth factor (IGF) binding protein complementary deoxyribonucleic acid from human HEP G2 hepatoma cells: predicted protein sequence suggests an IGF binding domain different from those of the IGF-I and IGF-II receptors. Mol Endocrinol 1988;2:404–411.
11 Grundmann U, Nerlich, C, Bohn H, Rein T. Cloning of cDNA encoding human placental protein 12 (PP12): binding protein for IGF-I and somatomedin. Nucleic Acids Res 1988;16:8711.
12 Workshop on IGF binding proteins, Vancouver, BC, Canada. June 17–19, 1989.
13 Alitalo T, Kontula K, Koistinen R, Aalto-Setälä IK, Julkunen M, Jänne OA, Seppälä M, de la Chapelle A. The gene encoding human low molecular weight insulin-like growth factor binding protein (IGF-BP25): regional localization to 7p12-p13 and description of a DNA polymorphism. Human Genetics 1989;83:335–338.
14 Koistinen R, Huhtala M-L, Stenman U-H, Seppälä M. Purification of placental protein PP12 from human amniotic fluid and its comparison with PP12 from placenta by immunological, physicochemical and somatomedin binding properties. Clin Chim Acta 1987;164:293–303.
15 Rutanen E-M, Pekonen F, Mäkinen T. Soluble 34K binding protein inhibits the binding of insulin-like growth factor I to its cell receptors in human secretory phase endometrium: evidence for autocrine/paracine regulation of growth factor action. J Clin Endocrinol Metab 1988;66:173–180.
16 Waites GT, James RFL, Bell SC. Immunohistochemical localization of the human endometrial secretory protein pregnancy-associated endometrial α_1-globulin, an insulin-like growth factor-binding protein, during the menstrual cycle. J Clin Endocrinol Metab 1988;67:1100–1104.
17 Wahlström T, Seppälä M. Placental protein 12 (PP12) is induced in the endometrium by progesterone. Fertil Steril 1984;41:431–434.
18 Rutanen E-M, Bohn H, Seppälä M. Radioimmunoassay of placental protein 12: levels in amniotic fluid, cord blood and serum of healthy adults, pregnant women and patients with trophoblastic disease. Am J Obstet Gynecol 1982;144:460–463.
19 Koistinen R, Stenman U-H, Alfthan H, Seppälä M. Highly sensitive time-resolved immunofluorometric assay for 34 KD somatomedin-binding protein. Clin Chem 1987;33:1126–1128.
20 Rutanen E-M, Seppälä M, Pietilä R, Bohn H. Placental protein 12 (PP12): Factors affecting levels in late pregnancy. Placenta 1984;5:243–248.
21 Baxter RC, Cowell CT. Diurnal rhythms of growth hormone-independent binding protein for insulin-like growth factors in human plasma. J Clin Endocrinol Metab 1987;65:432–440.
22 Hall K, Lundin G, Povoa G. Serum levels of the low molecular weight form of insulin-like growth factor binding protein in healthy subjects and patients with growth hormone deficiency, acromegaly and anorexia nervosa. Acta Endocrinol 1988;118:321–326.

23 Suikkari A-M, Koivisto VA, Rutanen E-M, Yki-Järvinen H, Karonen S-L, Seppälä M. Insulin regulates the serum levels of low molecular weight insulin-like growth factor-binding protein. J Clin Endocrinol Metab 1988;66:266–272.
24 Suikkari A-M, Koivisto VA, Koistinen R, Seppälä M, Yki-Järvinen H. Dose-response characteristics for suppression of low molecular weight plasma insulin-like growth factor-binding protein by insulin. J Clin Endocrinol Metab 1989;68:135–140.
25 Suikkari A-M, Jalkanen J, Koistinen R, Butzow R, Ritvos O, Ranta T, Seppälä M. Human granulosa cells synthesize low molecular weight insulin-like growth factor-binding protein. Endocrinology 1989;124:1088–1090.
26 Suikkari A-M, Rutanen E-M, Seppälä M. Circulating levels of immunoreactive insulin-like growth factor-binding protein in non-pregnant women. Hum Reprod 1987;2:297–300.
27 Singh A, Hamilton-Fairley D, Seppälä M, Koistinen R, Franks S, Reed MJ. The effect of insulin-like growth factor type I (IGF-I) and insulin of the secretion of sex hormone binding globulin and IGF-I binding protein by human hepatoma cells. J Endocrinol 1990;124:R1–3.
28 Seppälä M, Julkunen M, Koskimies A, Laatikainen T, Stenman U-H, Huhtala M-L. Proteins in the human endometrium: Basic and clinical studies toward a blood test for endometrial function. Ann NY Acad Sci 1988;541:432–444.
29 Suikkari A-M, Koistinen R, Koskimies AI, Seppälä M. Association of insulin-like growth factor-binding protein (IGF-BP25) with serum insulin and reproductive functions. In Drop SLS, Hintz RL, eds. Insulin-like growth factor-binding proteins. Amsterdam: Excerpta Medica, 1989:29–37.
30 Suikkari A-M, Ruutiainen K, Erkkola R, Seppälä M. Low levels of low molecular weight insulin-like growth factor-binding protein in patients with polycystic ovarian disease. Hum Reprod 1989;4:136–139.
31 Kiddy DS, Hamilton-Fairley D, Seppälä M, Koistinen R, James VHT, Reed MJ, Franks S. Diet-induced changes in sex hormone-binding globulin and free testosterone in women with normal or polycystic ovaries: correlation with serum insulin and insulin-like growth factor-I. Clin Endocrinol 1989;31:757–763.
32 Barbieri RL, Hornstein MD. Hyperinslulinemia and ovarian hyperandrogenism. Cause and effect. Endocrinol Metab Clin North Am 1988;17:685–703.
33 Iino K, Sjöberg J, Seppälä M. Elevated circulating levels of a decidual protein, placental protein 12, in pre-eclampsia. Obstet Gynecol 1986;68:58–60.
34 Howell RJS, Perry LH, Choglay NS, Bohn H, Chard T. Placental protein 12 (PP12): a new test for the prediction of the small-for-gestational-age infant. Br J Obstet Gynaecol 1986;92:1141–1144.
35 Iino K, Seppälä M, Heinonen PK, Sipponen P, Rutanen E-M. Elevated levels of a somatomedin-binding protein PP12 in patients with ovarian cancer. Cancer 1986;58:2294–2297.
36 Rutanen E-M, Wahlström T, Koistinen R, Sipponen P, Jalanko H, Seppälä M. Placental protein 12 (PP12) in primary liver cancer and cirrhosis. Tumor Biol 1984;5:95–102.
37 Seppälä M, Wahlström T, Koskimies AI, Tenhunen A, Rutanen E-M, Koistinen R, Huhtaniemi I, Bohn H, Stenman U-M. Human Preovulatory follicular fluid, luteinized granulosa cells of hyperstimulated preovulatory follicles, and corpus luteum contain placented protein 12. J Clin Endocrinol Metab 1984;58:505–510.
38 Bohn H, Kraus W, Winckler W. New soluble placental tissue proteins: their isolation, characterization, localization and quantification; immunology of human placental proteins. Placenta 1982;S4:67–81.
39 Julkunen M, Seppälä M, Jänne OA. Complete amino acid sequence of human placental protein 14; a progesterone-regulated uterine protein homologous to β-lactoglobulin. Proc Natl Acad Sci USA 1988;85:8845–8849.
40 Julkunen M, Raikar RS, Joshi SG, Bohn H, Seppälä M. Placental protein 14 and progestogen-dependent endometrial protein are immunologically indistinguishable. Hum Reprod 1986;1:7–8.
41 Sutcliffe RG, Joshi SG, Paterson WG, Bank JF. Serological identity between human uterine protein an human progestagen-dependent endometrial protein. J Reprod Fertil 1982;65:207–209.
42 Petrunin DD, Gryaznova IM, Petrunina YA, Tatarinov YS. Immunochemical identification of organ-specific human placental α_2-globulin and its content in the amniotic fluid (in Russian). Byull Eksp Biol Med 1976;82:803–804.
43 Huhtala M-L, Seppälä M, Närvänen A, Palomäki P, Julkunen M, Bohn H. Amino acid sequence

homology between human placental protein 14 and β-lactoglobulins from various species. Endocrinology 1987; 120:2620–2622.

44 Bell SC, Keyte JW, Waites GT. Pregnancy-associated endometrial α_2-globulin, the major secretory protein of the luteal phase and first trimester pregnancy endometrium, is not glycosylated prolactin but related to β-lactoglobulins. J Clin Endocrinol Metab 1987;65:1067–1071.

45 Seppälä M, Riittinen L, Julkunen M, Koistinen R, Wahlström T, Iino K, Alfthan H, Stenman U-H, Huhtala M-L. Structural studies, localization in tissue and clinical aspects of human endometrial proteins. J Reprod Fertil 1988;S36:127–141.

46 Seppälä M, Wahlström T, Julkunen M, Vartiainen E, Huhtala M-L. Endometrial proteins as indicators of endometrial function. In: Y Tomoda, S Mizutani, O Narita, A Klopper, eds. Placental and endometrial proteins: basic and clinical aspects. Utrecht: VNU Science Press, 1988;35–42.

47 Wood PL, Walker RA, Bell SC. Serum levels of pregnancy-associated endometrial α_2-globulin (α_2-PEG) during normal menstrual and combined oral contraceptive cycles and relationship to immunohistological localization. Hum Reprod 1989;4:140–146.

48 Julkunen M, Wahlström T, Seppälä M. Human fallopian tube contains placental protein 14. Am J Obstet Gynecol 1986;154:1076–1079.

49 Joshi SG, Bank JF, Henriques ES. Makarachi A, Matties G. Serum levels of a progestagen-associated endometrial protein during the menstrual cycle and pregnancy. J Clin Endocrinol Metab 1982;55:642–648.

50 Julkunen M, Apter D, Seppälä M, Stenman U-H, Bohn H. Serum levels of placental protein 14 reflect ovulation in nonconceptional menstrual cycles. Fertil Steril 1986;45:47–50.

51 Julkunen M, Rutanen E-M, Koskimies AI, Ranta T, Bohn H, Seppälä M. Distribution of placental protein 14 in tissues and body fluids during pregnancy. Br J Obstet Gynaecol 1986;92:1145–1151.

52 Riittinen L, Stenman U-H, Alfthan H, Suikkari A-M, Bohn H, Seppälä M. Time-resolved immunofluorometric assay for placental protein 14, Clin Chim Acta 1989;183:115–124.

53 Riittinen L, Julkunen M, Seppälä M, Koistinen R, Huhtala M-L, Purification and characterization of endometrical protein PP14 from mid-trimester amniotic fluid. Clin Chim Acta 1989;184:19–30.

54 Seppälä M, Martikainen H, Rönnberg L, Riittinen L, Kauppila A. Suppression of prolactin secretion during ovarian hyperstimulation is followed by elevated serum levels of endometrial protein PP14 in the late luteal phase. Hum Reprod 1989;4:389–391.

55 Joshi SG, Rao R, Henriques EE, Raikar RS, Gordon M, Luteal phase concentration of a progestagen-associated endometrial protein (PEP) in the serum of cycling women with adequate or inadequate endometrium. J Clin Endocrinol Metab 1986;63:1247–1249.

56 Seppälä M, Rönnberg L, Karonen S-L, Kauppila A. Micronized oral progesterone increases the circulating level of endometrial secretory PP14/β-lactoglobulin homologue. Hum Reprod 1987;2:453–455.

57 Telimaa S, Kauppila A, Rönnberg L, Seppälä M. Elevated serum levels of endometrial secretory protein PP14 in patients with advanced endometriosis: suppression by treatment with danazol and high dose medroxyprogesterone acetate. Am J Obstet Gynecol 1989;161:866–871.

58 Seppälä M, Alfthan H, Vartiainen E, Stenman U-H. The postmenopausal uterus: The effect of hormone replacement therapy on the serum levels of secretory endometrial protein PP14/β-lactoglobulin homologue. Hum Reprod 1987;2:741–743.

59 Bolton AE, Pockley AG, Clough KJ, Mowles EA, Stocker RJ, Westwood OMR, Chapman MG. Identification of placental protein 14 as an immunosuppressive factor in human reproduction. Lancet 1987;i:593–595.

60 Check JH, Joshi S, Vaze M, Vetter B. Measurement of serum progestagen-associated endometrial protein (PEP) not helpful in predicting infertility on spontaneous abortion. European Society of Human Reproduction, 5th Annual Meeting, June 25–28, 1989. Malmö, Sweden. Abstr No. T6:2.