ESTRADIOL AND PROGESTERONE BINDING IN UTERINE LEIOMYOMATA AND PREGNANT MYOMETRIUM

G. B. NARDELLI - M. MEGA - M. BERTASI - F. SILIOTTI
Institute of Obstetrics and Gynaecology - University of Padua (Italy)

Summary: In order to verify the endogenous steroid influences on the myometrium, we compared the receptor status in 18 specimens of uterine leiomyomata and 31 specimens of pregnant myometrium.

17-β-Estradiol and Progesterone receptors were assayed in the cytosol and nuclear extracts. In the uterine leiomyomata we observed the following mean values: PgR/c 279 fmol/mg, PgR/n 89 fmol/mg, ER/c 52.5 fmol/mg, ER/n 12.3 fmol/mg. In the pregnant myometrium we observed the following mean values: PgR/c 5.86 fmol/mg, PgR/n 166 fmol/mg, ER/c 0.76 fmol/mg, ER/n 1.65.

It was concluded that direct correlation between estrogen power and progesterone receptor replenishment in the nucleus exists.

The myometrium is influenced by the ovary steroids, which translocate cytoplasmatic receptors to the nucleus for gene expression; this explains the sensitivity of the uterus to endogenous steroids. Little is known about the interactions of the hormones and their action mechanism in benign uterine tumors.

The fibroid tumors of the human uterus bind more than 20% of estradiol than the normal myometrium ([1]). Other Authors observe no difference in the uptake of estrogens by leiomyomata and normal myometrium ([2]), or in corresponding receptor contents which were observed in the normal myometrium and endometrium of the same uterus ([3]).

Anyway, the ER of the myoma had the physicochemical and steroid-binding characteristics similar to the corresponding receptors in a normal human myometrium ([4, 5]). Tamaya ([6]) observed ER levels higher in leiomyomata than in a normal myometrium.

The purpose of this report is to confirm ([7, 8, 9, 10, 11, 12, 13, 14, 15]) the different myometrium modulations steroid-induced through estrogen and progesterone receptor assay in the cytoplasm and the nucleous.

MATERIAL AND METHODS

49 portions of uterine tissue were obtained from surgical specimens of 18 patients who had undergone hysterectomy for uterine leiomyomata and 31 pregnant women who had undergone cesarean section for twin pregnancy, anomalies of engagement and positioning of the fetal head and antepartum hemorrhage.

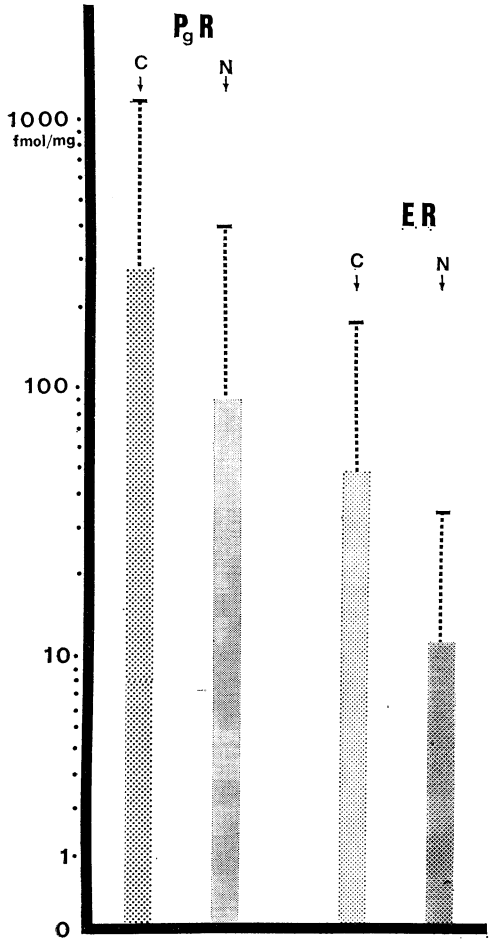
Approximately 10 gms of myometrium was obtained immediately after surgery and stored in liquid nitrogen until processing. The concentrations of estrogen and progesterone receptors were obtained by dextran-coated-charcoal (DCC) ([16]).

Preparation of tissue and subcellular fractions: The frozen tissues stored in N_2 liquid were weighed and pulverized in microdismembrator-Braun-852 062 Melsungen AG or in Mortar AISI 304, accurately frozen beforehand. The powder was then transferred into prechilled glass tubes of 20×34 mm and transferred to the cold-room at 4 °C. All steps of the processing were carried out in the cold-room to assure a constant reaction temperature. The powder was suspended in buffer in a w/v ratio and homogenized with Politron-Brinkman PT 10 ST by three 10 sec. bursts at the very lowest setting. The homogenized material was carefully transferred with a spatula into polycarbonate ultracentrifuge tubes with a screw cap (Kontron 13.5 ml, 5/8″×3″, 16×76 mm). The homogenate was centrifuged at 105,000 g/60 min with Kontron Rotor TFT 65.13 in Kontron TGA 65.

The cytosol was aspirated with cold Pasteur and after having carried out the estimate of the nucleic acids ([17]) was suitably diluted.

200 λ of sample, in duplicate, were incubated in the cold-room with 1 hour intervals with the

Table 1. — *Cytoplasmatic and nuclear ER-PgR concentrations in the uterine leiomyomata tissues.*



respective competitors, then for 16 hrs with radiolabelled steroids. DCC 1% - BSA was added to each tube and, after 15 min of vigorous shaking into Varvel FVF 20, the tubes were put into the Beckman TJ6 centrifuge at 3,000 rpm/15 min/4 °C. A 500 λ aliquot was read in a Kontron-Betamatic I - β Counter with an efficiency of 57%. The sparking liquid was prepared with Toluol-PPO-POPOP.

Saturation analysis: We used 4.8 ml of cytosol for each receptor assay to perform six points in duplicate. A saturation analysis and successive corrections of the Scatchard-Plot [18] biphasic according to Chamness-McGuire [19] were carried out through the DPM data transferred on Cardio-2145665-Kontron desk computer by Jawny's software [20].

The Scatchard analysis did not reveal any changes in the affinities of estrogen and progesterone receptors in cytosols assayed in pregnant or non pregnant women. The PgR equilibrium dissociated constant was 4.23 ± 0.05 nM and 0.59 ± 0.05 nM for ER in cytosol and 0.6 M KCl-extractions. The statistical analysis was carried out by IBM-PC-XT desk computer and the values were compared by the Student' test.

Steroids and buffers: We used the following steroid concentrations:

Promegestone ^{3}H-R5020 (NEN) ranged between 2.94×10^{-10} M - 8.4×10^{-12} M;

Table 2. — *Cytoplasmatic and nuclear ER-PgR concentrations in pregnant uterine specimens.*

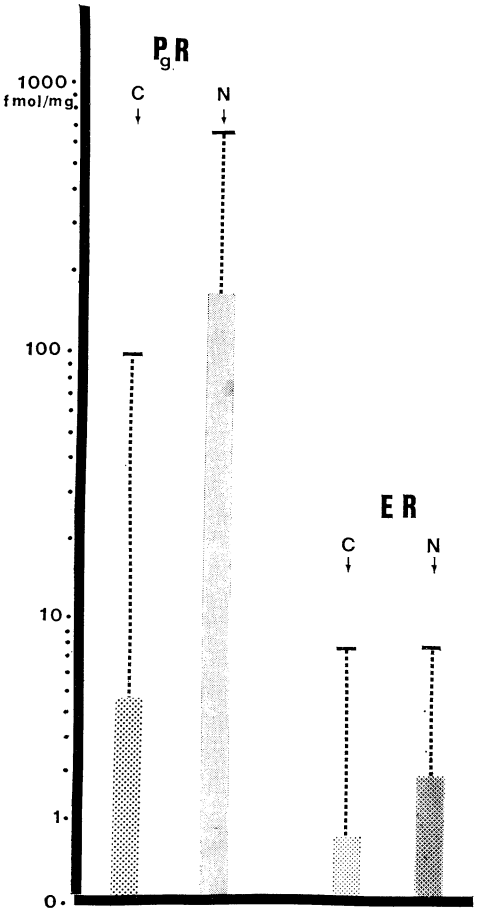
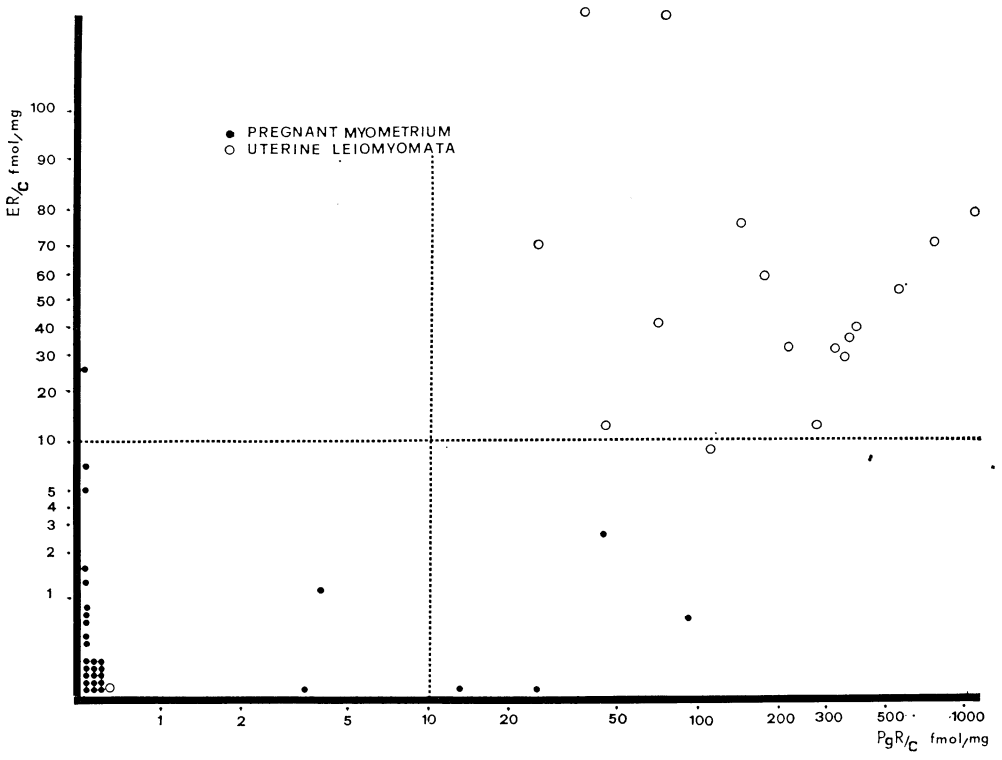


Table 3. — *ER vs PgR distribution in the cytoplasm of pregnant myometrium (full circle) and in uterine leiomyomata (empty circle). We used 10 fmol/mg as positive cut-off.*



Promegestone R5020-Cold (NEN) ranged between 2.94×10^{-8} - 8.4×10^{-10} M;

2,4,6,7 ^{3}H Oestradiol (Amersham) ranged between 2.28×10^{-11} M - 1.5×10^{-12} M;

DES (Sigma Ch.) ranged between 2.28×10^{-9} M - 1.5×10^{-10} M;

5 α Dihydrotesterone (Sigma Ch.) and Hydrocortisone Acetate (F) (Calbiochem-Behring Co.) ranged between 7.5×10^{-8} M - 2.3×10^{-9} M.

We used the following chemicals in buffers: TRIS 0.01 M (Schwartz/Mann Ultra pure), EDTA 0.0015 M (Fischer Sc. Co.), Molybdate 5 mM (Sigma Co.), 12 N HC L pH 7.4/4 °C;

DTT-Dithiothreitol 0.5 mM (Calbiochem-Behring Co.);

Dextran Grade C 0.025 gms/l (BDH Ch.), Charcoal Activated 2.5 gms/l, Albumina Bovine-Fraction V 1% (Sigma Co.).

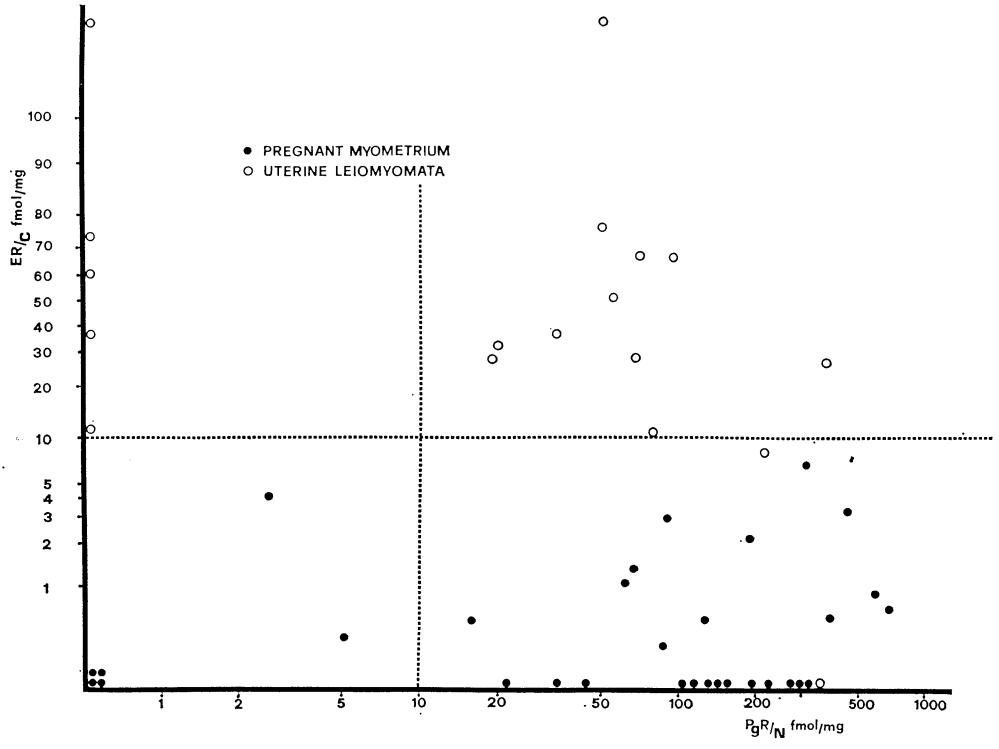
Nuclear receptors: The pellets previously homogenized in TEDMo-Buffer were resuspended in 0.6 M-KCl-Buffer and homogenized with a teflon pestle directly in the polycarbonate tubes. They were then shaken gently for 30 min in cold-room/4 °C on Varvel FVF 20 and afterwards they were centrifuged at 105,000 g/30 min. After the standardization of the proteic concentration with the estimate of the nucleic acids, the cytosol was suitably diluted; the samples, in duplicate, were incubated with the modality of the cytoplasmatic receptors.

Protein assay: The nucleic acids were assayed according to Layne ([17]) by ultraviolet absorption at 260-280 nm in 20 λ of sample. The proteins were assayed according to Lowry's Method on the LBC-Ultrospec-4050-Spectrophotometer and ([21]) results were expressed in fmol/mg. The protein concentrations ranged in 2.0-4.0 mg/ml for PgR and 0.75-2.0 mg/ml for ER. We considered 10 fmol/mg the positive cut-off.

RESULTS

Uterine leiomyomata: Table 1 shows the cytoplasmatic and nuclear ER and PgR concentrations in uterine leiomyomata.

Table 4. — *ER vs PgR distribution in the nucleus of pregnant myometrium (full circle) and in uterine leiomyomata (empty circle). We used 10 fmol/mg as positive cut-off.*



The cytoplasmatic range was 0-1144 fmol/mg of protein (m.v. 279 fmol/mg) with positive values (more than 10 fmol/mg) in 94% of the cases. The nuclear PgR range was 0-378 fmol/mg of protein (m.v. 89 fmol/mg) with a 72% of positive values.

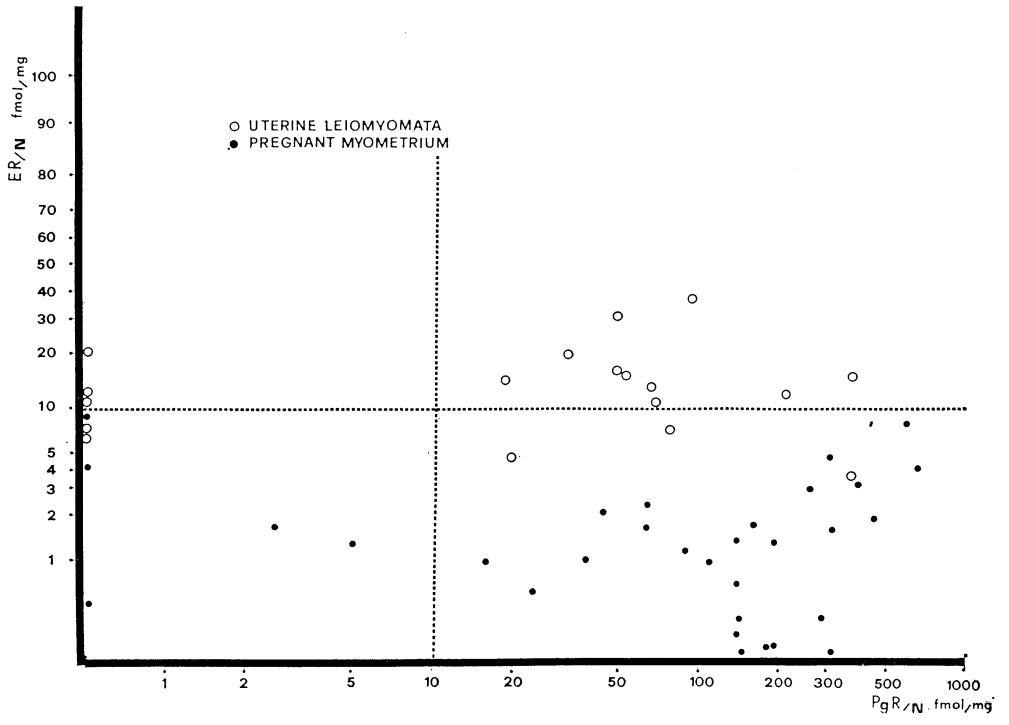
The cytoplasmatic ER range was 0-178 fmol/mg of protein (m.v. 52.5 fmol/mg) with a positive value (more than 10 fmol/mg) in 88.8% of the cases. The nuclear PgR range was 3-27 fmol/mg of protein (m.v. 12.3 fmol/mg) with a 50% of positive values.

Pregnant myometrium: The cytosolic PgR range was 0-98 fmol/mg of protein (m.v. 5.86 fmol/mg) with positive levels (more than 10 fmol/mg) in 13% of the cases. The nuclear PgR range was 0-659 fmol/mg of protein (m.v. 166 fmol/mg) with a 77% of positive values (table 2).

The cytosolic ER range was 0-7.5 fmol/mg of protein (m.v. 0.76 fmol/mg) and the nuclear ER range was 0-7.8 fmol/mg (m.v. 1.65 fmol/mg). No ER/c or ER/n positive cases (more than 10 fmol/mg) were observed.

Logit-Log plotting analysis: The Logit-Log plotting allows us to perform the analysis of the cytoplasmatic and nuclear steroid receptors replenishment, with regard to different hormonal influence. Table 3 shows the ER/c distribution as to PgR/c of the myometrium in pregnancy (full circle) and pre-early menopause (empty circle). In the uterine leiomyomata (U.L.)

Table 3. — *Cytoplasmatic ER vs nuclear PgR distribution in pregnant myometrium (full circle) and in uterine leiomyomata (empty circle). We used 10 fmol/mg as positive cut-off.*



we observed ER/c and PgR/c high positive levels (more than 10 fmol/mg of protein) in 88.8% of the cases, while in the pregnant myometrium (P.M.) we observed high levels (more than 10 fmol/mg of protein) of PgR/c in 9.6% and ER/c in 3.2% of the cases.

Table 4 shows the ER/n distribution as to PgR/n in P.M. (full circle) and in U.L. (empty circle). In the pregnant myometrium we observed a relationship between high PgR/n levels and ER/n concentrations lower than 10 fmol/mg. In the uterine leiomyomata instead, there are high PgR/n levels related to ER/n concentrations above than 10 fmol/mg. In order to verify whether a relationship between ER/n and PgR/n exists, we plotted the cytoplasmatic ER versus the nuclear PgR values (table 5). The results confirm again that the final product of estrogen action (PgR/n) in the P.M. is function of low ER/c levels; on the contrary, in the U.L. is a function of high ER/c levels.

DISCUSSION

The two myometrium types reflect a different way of hormonal stimulation. The receptorial status of uterine leiomyiomata in pre-early menopause in the main depends on strong estrogen (estradiol) with an intense mitotic activity and high protein synthesis. The receptorial turnover is faster according to higher progesterone receptor concentrations in the cytoplasm and the nucleus. The control mechanism of the myometrium at the end of pregnancy reflects the delicate balance among the estradiol power and certain metabolic de-

rivates (estrone, estriol). The estrogen exerts its effect by binding first to a cytoplasmatic receptor to form an ER complex; this complex undergoes translocation to the nucleus stimulating the RNA synthesis and cell growth.

The progesterone, reducing ER synthesis, antagonizes the estrogen effects by changing the estrogen power, and changes the antagonizing progesterone action. In pregnancy many estrogens act, with different mechanisms; consequently the progesterone interferes with the replenishment of the cytoplasmatic estrogen receptor and this reduction is related to a reduced sensitivity of the uterus to estrogen. The uterine leiomyomata showed ER/c concentrations higher than ER/n in concomitance with PgR/c levels higher than PgR/n. These data suggest that ER/c replenishment may be accomplished by estradiol, characterized by diminuished nuclear retention time.

The pregnant myometrium showed an ER and PgR cytoplasmatic concentrations always lower than the nucleus. These data suggest that the long-term nuclear retention of ER/n is related to the stimulation of true uterine growth. So, the unexpected but exact observation that the highest PgR/n concentrations in the pregnant myometrium exist, could explain the ability of progesterone to antagonize and/or modify the action of the high levels of weak estrogens. In fact we observed cytoplasmatic receptor levels higher in non-pregnant than in pregnant myometrium. Moreover cytoplasmatic and nuclear ER > 10 fmol/mg are related with a PgR/n synthesis greater in the pregnant myometrium than in the uterine leiomyomata; ER > 10 fmol/mg are related to a PgR/n synthesis only in uterine leiomyomata.

CONCLUSIONS

The myometrium shows different receptorial status according to strong or weak estrogen stimulation. In our experience the correlation between estrogen blood levels and their receptors does not exist, but we observed a direct correlation between estrogen power and progesterone receptor replenishment in the nucleus.

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