Patterns of protein synthesis in endometrial tissues from ovariectomized rats treated with oestradiol and progesterone

B. Lejeune, F. Lamy*, R. Lecocq*, J. Deschacht and F. Leroy

*Human Reproduction Research Unit and *Institute for Interdisciplinary Research,
School of Medicine, Free University of Brussels, 1000 Brussels, Belgium*

Summary. Groups of ovariectomized rats were taken as controls or given hormonal treatment mimicking the successive steps in the sequence of ovarian secretions leading to implantation. Total endometrium or separated epithelium and stroma were incubated *in vitro* with [³⁵S]methionine. Dissolved proteins were submitted to two-dimensional polyacrylamide gel electrophoresis (pH range 5–7), followed by autoradiography. Priming with oestradiol (2 days) and subsequent treatment with progesterone (3 days) enhanced the synthesis of 12 and 14 polypeptides, respectively, which are specific for each of these treatments. Progesterone also suppressed the production of 10 oestrogen-dependent proteins both in the epithelium and the stroma. When an oestrogen–progesterone–oestrogen treatment was given, synthesis of all but 4 of the progesterone-induced polypeptides in the epithelium was inhibited while 5 of the proteins abolished by progesterone in this tissue compartment reappeared. These results are compatible with a mechanism of implantation acting at the epithelial level by lifting of intrauterine inhibition and stimulation by embryotrophic substances.

Introduction

Rat blastocysts can implant only after the rise of oestrogen secretion occurring between Days 3 and 4 of gestation (Psychoyos, 1973). In animals ovariectomized on Day 3 and treated with progesterone, implantation does not take place as long as no oestrogen is provided (McLaren, 1973).

Two explanations for the nidatory action of oestrogen have been proposed: (1) stimulation of the synthesis of specific factors which induce blastocyst activation and attachment, and (2) inhibition of the synthesis of intrauterine substances which prevent implantation. Supporting the first hypothesis are the increased uterine RNA and general protein synthesis on Day 5 (Heald, 1976), the induction of implantation by uterine RNA extracts (Segal, Scher & Koide, 1977; Lejeune, Puisant, Camus & Leroy, 1982b) and the secretion into the uterine lumen of specific proteins at Day 5 (Surani, 1977). On the other hand, stimulation of implantation by actinomycin D (Finn, 1974; Camus, Lejeune & Leroy, 1979) as well as the inhibitory influence on blastocysts of fluids from uteri of animals with delayed implantation (Psychoyos, 1973; Weitlauf, 1976, 1978; O'Neill & Quinn, 1981, 1983), stand in favour of a mechanism acting through release from inhibition.

In rats and mice, implantation depends on a sequence of ovarian secretions (oestrogen, progesterone and oestrogen) of which each component fulfils a particular role (Finn & Martin, 1974; Leroy, Schetgen & Camus, 1980). Therefore, the aim of the present study was to analyse changes in the pattern of endometrial protein synthesis in relation to implantation, by experiments mimicking the progestational period (Psychoyos, 1961; Finn & Martin, 1974), and to study the effects of each of its hormonal components on whole endometrium or on separated epithelium and stroma.

Materials and Methods

At 2 weeks after ovariectomy groups of 10 Wistar rats (body weight 150 g) were taken as controls (Group 1) or given one of the following sequential treatments: 1 µg oestradiol-17β daily for 2 days (Group 2); 1 µg oestradiol-17β for 2 days, 2 days of rest and 5 mg progesterone per day for 3 days (Group 3); 1 µg oestradiol-17β for 2 days, 2 days of rest, 5 mg progesterone for 3 days and 50 ng oestradiol-17β in the evening of the third day of progesterone treatment (Group 4).

Animals were killed 24 h (Groups 2 and 3) or 15 h (Group 4) after the end of hormonal treatment. Uteri from Groups 1 and 2 were slit lengthwise and total endometrium was scraped off with a scalpel. Pure epithelium was squeezed out of uterine horns from progesterone-treated animals (Groups 3 and 4) by use of forceps (Bitton Casimiri, Rath & Psychoyos, 1977). The horns were then opened longitudinally and the remaining stroma was removed by scraping.

The endometrial tissues were incubated for 15 min at 37°C in 10 ml Krebs–Ringer–bicarbonate solution (KRB) (Krebs, 1950) supplemented with 20 mg collagenase (clostridiopeptidase A; Böehringer, Mannheim). After three washes in KRB the tissues were centrifuged for 10 min at 200 g and incubated for 8 h at 37°C under an atmosphere of 5% CO₂–95% O₂ in 1 ml Eagle's medium (Flow Labs, Brussels) supplemented with 500 µCi [³⁵S]methionine (sp. act. 800 Ci/mmol; Amersham, Belgium). Protein sample preparations, measurements of incorporated radioactivity, two-dimensional gel electrophoresis according to O'Farrell (1975) and autoradiography, were performed as described in a previous study (Lejeune, Lecocq, Lamy & Leroy, 1982a).

Results

Three separate experiments, each including the four groups described above, gave almost identical results indicating that the effects of the different hormone treatments were reproducible. Prominent modifications of polypeptide synthesis are listed in Table 1. Approximate molecular weights and isoelectric points under the adopted working conditions were calculated as previously described (Lejeune *et al.*, 1982a). Oestradiol given alone for 2 days (Group 2) promoted or increased the synthesis of 12 polypeptides (numbered from 1 to 12 on Pl. 1, Fig. 2) of relatively low molecular weight (23 000–55 000) which were not detectable in the endometrium of ovariectomized rats

PLATES 1–3

Autoradiographic patterns of two-dimensional electrophoretic separation of [³⁵S]methionine-labelled proteins, extracted from rat endometrium sequentially treated with oestradiol, progesterone and oestradiol. Polypeptides of which the synthesis is either markedly enhanced or reduced as compared to the previous treatment group are indicated by arrows or pointed triangles, respectively.

PLATE 1

Fig. 1. Total endometrium from ovariectomized animals (Group 1).

Fig. 2. Total endometrium from animals primed with oestradiol (Group 2).

PLATE 2

Fig. 3. Luminal epithelium from animals treated with oestradiol plus progesterone (Group 3).

Fig. 4. Endometrial stroma from animals treated with oestradiol plus progesterone (Group 3).

PLATE 3

Fig. 5. Luminal epithelium from animals treated with oestradiol, progesterone and oestradiol (Group 4).

Fig. 6. Endometrial stroma from animals treated with oestradiol, progesterone and oestradiol (Group 4).

PLATE 1

(Facing p. 224)

PLATE 2

PLATE 3

Table 1. Analysis of the effects of oestradiol (E₂) and progesterone (P) on endometrial protein synthesis by two-dimensional electrophoresis

Polypeptide no.	Approximate mol. wt ($\times 10^{-3}$)	Approx. isoelectric point	Treatment groups				
			Group 2 (E ₂)	Group 3 (E ₂ + P)		Group 4 (E ₂ + P + E ₂)	
			Total endometrium	Epithelium	Stroma	Epithelium	Stroma
1	22	5.67	+	—	—	0	0
2	29	6.22	+	—	—	+	+
3	30	5.40	+	—	—	+	0
4	36	6.62	+	0	0	0	0
5	37	6.62	+	—	—	+	0
6	37	6.37	+	0	0	0	0
7	43	5.88	+	—	—	0	0
8	43	5.76	+	—	—	0	0
9	44	6.00	+	—	—	+	0
10	52	6.33	+	—	—	0	0
11	55	6.14	+	—	—	0	0
12	55	6.28	+	—	—	+	0
13	23	5.55		+	+	—	0
14	30	5.50		+	+	0	0
15	32	6.14		+	+	0	0
16	35	5.48		+	0	—	0
17	49	6.20		+	+	—	0
18	53	5.86		+	0	—	0
19	54	5.44		+	0	—	+
20	55	5.86		+	+	—	—
21	72	6.10		+	0	0	0
22	75	6.10		+	+	0	0
23	80	6.24		+	0	—	0
24	80	6.24		+	0	—	0
25	90	5.80		+	0	—	0
26	105	5.78		+	0	—	0

For easy comparison, polypeptides are classified by increasing molecular weight in 2 successive series (1–12 and 13–26).

+, newly appearing or markedly increased synthesis; —, disappearing or markedly reduced synthesis; 0, not modified by comparison with previous treatment group.

(Group 1). No protein synthesized by unstimulated endometrium disappeared after oestrogen treatment. By contrast, 3 days of progesterone treatment given after oestradiol priming followed by 2 days of rest (Group 3) caused the disappearance of 10 of the oestrogen-stimulated polypeptides (Nos 1, 2, 3, 5, 7, 8, 9, 10, 11 & 12 on Pl. 2, Figs 3 & 4) in the epithelium as well as in the stroma. However, progesterone induced the synthesis of 14 new polypeptides (numbered from 13 to 26 on Pl. 2, Fig. 3) in the epithelium (mol. wt 23 000–105 000) amongst which 6 were also detected in the stromal fraction (Nos 13, 14, 15, 17, 20 & 22 on Pl. 2, Fig. 4). Injection of 50 ng oestradiol on the 3rd day of progesterone treatment (Group 4) resulted in the inhibition of all but 4 (Nos 14, 15, 21 & 22) of the polypeptides induced by progesterone in the epithelial compartment. Moreover, 5 epithelial proteins (Nos 2, 3, 5, 9 & 12) for which synthesis was abolished by progesterone (Group 3) reappeared (Pl. 3, Fig. 5). In the stroma, however, very few differences were observed: spots 2 and 19 were increased while No. 20 was decreased (Pl. 3, Fig. 6).

Discussion

Oestrogen administration to ovariectomized or immature animals entails notable amplification of protein synthesis by uterine cells while many enzyme activities become increased at this level (see

Segal *et al.*, 1977). Therefore, it is not surprising that after 2 days of priming with oestradiol we could observe the synthesis of at least 12 peptides which were not detectable in the endometrium of ovariectomized rats.

Much work has been devoted to the identification of protein synthesis which is specifically elicited through oestrogen treatment. These studies, however, cannot be validly compared to our results since they mostly deal with the so-called 'induced protein' (IP) which can only be detected during the first few hours of oestrogen action (De Angelo & Gorski, 1970; Cohen & Hamilton, 1975). Identification of acidic nuclear proteins, the synthesis of which is specifically stimulated by oestradiol, is likewise related to durations of hormone action much shorter than in the present work (Barker, 1971; Hamilton, 1971). Also the analysis of uterine protein synthesis by a two-dimensional method as carried out by Korach, Harris & Carter (1981) did not extend beyond 7 h after oestradiol administration.

By use of monodimensional gel electrophoresis after the same priming treatment with oestradiol as in our experiments, Surani (1977) was able to show that the profile of uterine protein synthesis closely resembles that observed at normal pro-oestrus. Labelled proteins of high molecular weight (80 000–135 000) are mainly represented under such conditions. The absence of ovariectomized controls in this study makes it difficult to draw a close comparison with our data. However, the relative paucity of labelled proteins of high molecular weight in our experiments may be explained by the more denaturing technical conditions that we used, i.e. treatment with 8 M-urea and mercaptoethanol.

Compared to the effects of oestrogen given for 2 days, long-term administration of progesterone results in relatively low radiolabelling of rat uterine luminal proteins (Surani, 1977). When injected at different times after priming with oestradiol, a single dose of progesterone is able to inhibit overall uterine protein synthesis within 2 h (Trams, Brewitt, Möllmann & Maas, 1973). Similar antagonistic effects of progesterone administration were found in relation to several specific responses to oestradiol. Pretreatment with progesterone inhibits subsequent oestrogen-dependent induced protein synthesis (Bhakoo & Katzenellenbogen, 1977). Given after priming with oestradiol, progesterone prevents the oestrogen-receptor replenishment which is normally induced by oestrogen (Hsueh, Peck & Clark, 1976). However, oestrogen-stimulated synthesis of sulphonated glycoproteins in the luminal epithelium of rat uterus becomes arrested through subsequent progesterone administration (Takata & Terayama, 1979). Our observation that progesterone treatment inhibits the production of most polypeptides which are induced by priming with oestradiol is consistent with the above-mentioned antagonistic effects. However, under our experimental conditions, which closely mimic the progestational hormonal sequence (Finn & Martin, 1974), progesterone redirected endometrial protein synthesis by initiating the appearance of 14 new peptides in the epithelium and 6 in the stroma. Only 2 labelled compounds remained common to oestradiol priming and subsequent progesterone stimulation. Our results show that progesterone tends to switch endometrial protein synthesis from oestrogen-dependent low molecular weight substances to the production of bigger polypeptides. This observation is compatible with the hypothesis that progesterone prepares the endometrium for blastocyst implantation by first eliciting gene activation, transcription and translation of chromosomal information (O'Malley, 1971).

The addition of 50 ng oestradiol to oestrogen and progesterone priming (Group 4) suppressed the production of 10 epithelial peptides and 1 stromal peptide. Since small amounts of oestrogen are able to trigger the attachment of delayed blastocysts (Psychoyos, 1973), these changes would support the theory that implantation occurs after the disappearance of intrauterine inhibitory factors (Psychoyos, 1973; Finn, 1974; Leroy *et al.*, 1980). No synthesis of any new peptide was observed in Group 4 animals. Therefore, our results tend to contradict the proposition that blastocyst activation depends on the production of a molecule which inactivates inhibitory proteins (Weitlauf, 1976). Such a factor, however, might originate from the embryos themselves (O'Neill & Quinn, 1981, 1983). In a recent study using bidimensional mapping it was found that in the endometrium of pregnant rats killed at the time of implantation (Day 5) no unique protein appears while several peptides are no longer synthesized (Mulholland & Villet, 1984).

It is possible that implantation is related to intrauterine release of embryotrophic substances (Aitken, 1977; Surani, 1977). In this context we found that 4 proteins synthesized in the progesterone-dominated epithelium (Group 3) remain present after 'nidatory' oestrogen action (Group 4). In view of their similar molecular weights (30 000–75 000), these peptides might belong to the group described by Surani (1977) in uterine flushings obtained at Day 5 of pregnancy or after combined progesterone and oestradiol treatment. These compounds, which are already synthesized in the progesterone-treated epithelium, as shown in our work, might be secreted into the uterine lumen only after treatment with 'nidatory' oestrogen. Since flushing has been shown to disrupt the endometrial lining it is also possible that more intracellular proteins are liberated as a consequence of increased damage which occurs when tight closure of the uterine lumen has been completed through luteal oestrogen action (Martin, 1984; Milligan & Martin, 1984).

Five proteins (Nos 2, 3, 5, 9 & 12) (mol. wt 29 000–55 000) induced by oestradiol given alone but disappearing when there is additional progesterone treatment are again synthesized after giving 'nidatory' oestrogen. These findings are consistent with results showing that implantation of delayed blastocysts can be obtained by injecting uterine RNA extracts from oestrogen-treated ovariectomized rats (Segal *et al.*, 1977; Lejeune *et al.*, 1982b).

Very few modifications occur in the stroma while nearly all appearing and disappearing polypeptides are observed in the uterine epithelium after 'nidatory' oestrogen administration. Such observations support the theory that luteal oestrogens are mainly acting at the level of the epithelial cells (Finn, 1974; Leroy *et al.*, 1980) in the triggering of implantation.

On the whole, our data are compatible with the hypothesis that implantation is contingent upon 2 chains of transcriptional and translational events (Leroy *et al.*, 1980), i.e. (1) intraluminal secretion of inhibitory proteins controlled by progesterone and of which the synthesis can be suppressed by luteal oestrogen or by actinomycin D, and (2) production of embryotrophic proteins (perhaps already present in ovariectomized animals) which is enhanced by oestrogen at pro-oestrus and able to overcome the repressive effects of progesterone-dependent inhibitors if overstimulated by exogenous uterine RNA.

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