Synthesis of polypeptides by the cervix of the baboon
(Papio anubis)*

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Summary. Administration of oestradiol to ovariectomized baboons caused the epithelium of the cervix to differentiate into tall columnar cells that were ciliated or secretory. Administration of progesterone in the presence or absence of oestradiol altered the appearance of the lining epithelium, suggesting a decrease in secretory activity. Fluorographs of media from cultures of tissue from steroid-treated animals reflected changes in polypeptide biosynthesis which correlated with the morphological observations: 6 polypeptides (Mr 88 000–37 000; pI 5.5–6.0) were observed in all treatment groups and, except for relative changes in intensity, these polypeptides were electrophoretically similar to those synthesized by the endometrium. A new group of low molecular weight polypeptides (Mr 23 000–20 000, pI > 8.0–5.5) and a basic protein (Mr 160 000) were synthesized and released in the oestradiol-dominated animal. These polypeptides were distinct to the cervical mucosa since they were not observed in the endometrium or oviduct. Progesterone suppressed the synthesis of the low molecular weight acidic polypeptides (Mr 23 000–20 000; pI 6.1–5.5) but maintained the synthesis of the basic polypeptides (Mr 23 000–20 000; pI > 8.0). Treatment with progesterone ± oestradiol did not appear to induce the synthesis of any new major polypeptides in the cervical epithelium. These results suggest that oestradiol induces the synthesis of a group of cervix-specific polypeptides and progesterone antagonizes the action of oestradiol in the baboon cervix.

Keywords: baboon; cervix; proteins; synthesis; morphology

Introduction

Cervical mucus has been purified (van Kooij et al., 1980) and the changes it undergoes during a normal menstrual cycle studied (van Kooij & Kramer, 1982). However, little is known about the possible synthesis and release of other steroid-dependent macromolecules by the primate cervical mucosa. The major objective of this study was to determine whether steroid-dependent polypeptides were synthesized and released during the culture of baboon endocervical explants and to compare this synthetic activity with that occurring in the oviduct (Fazleabas & Verhage, 1986; Verhage & Fazleabas, 1988) and uterus (Fazleabas & Verhage, 1987; Fazleabas et al., 1988) from the same animals. Additionally, steroid-induced morphological changes were documented. Comparisons between cervical, oviducal and endometrial polypeptide secretory patterns could enhance our understanding of how the functions of these organs are co-ordinated by endocrine signals.

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Materials and Methods

Materials. Tissue culture supplies were purchased from Grand Island Biological Co. (Grand Island, NY, USA) and electrophoretic supplies were obtained from Bio-Rad Laboratories (Richmond, CA, USA). Ampholines were purchased from LKB (Uppsala, Sweden). 1-[\(^{35}\)S]methionine (sp. act. 1142 Ci/mmol) was obtained from New England Nuclear (Boston, MA, USA). All other inorganic chemicals of reagent grade or better were products of Sigma Chemical Co. (St Louis, MO, USA).

Intact animals. The cervix was obtained from 4 mature cycling baboons (Papio anubis) at laparotomy. Each animal was designated as being in the early or late follicular, or mid- or late luteal phase (1 animal at each stage) based on previous menstrual history, last menses, sex tumescence, visual observation of the ovaries at the time of laparotomy and histology of the oviduct.

Steroid-treated animals. Mature baboons (Papio anubis) were ovariectomized via a mid-ventral laparotomy. At least 60 days later the animals were divided into 7 groups: 3 animals were left untreated and served as the ovariectomized control group. The remaining 12 animals (2 animals/group) were treated with oestradiol or primed with oestradiol and then treated with progesterone in the presence or absence of oestradiol according to the schedule presented in Fig. 1 and described in detail previously (Fazleabas et al., 1988; Verhage & Fazleabas, 1988).

![Experimental design for steroid treatment](image)

Fig. 1. Experimental design for steroid treatment. Silastic capsules (6 cm) filled with oestradiol-17\(\beta\) (E\(_2\)) or progesterone (P\(_4\)) were inserted s.c. in the mid-scupular region of the ovariectomized baboon. Oestradiol was administered for 7 days (one oestradiol implant) or 14 days (two oestradiol implants for the last 7 days). All progesterone-treated animals were primed with this 14-day oestradiol protocol before administration of progesterone in the presence of one oestradiol implant (3 progesterone implants) or in the absence of oestradiol (2 progesterone implants).

Steroids were administered via 6-cm steroid-filled Silastic capsules (Dow Corning, Midland, MI, USA; sp601-335) placed s.c. in the mid-scupula region. The concentration of oestradiol and progesterone were determined by radioimmunoassay (Fazleabas et al., 1988).

Morphology. Immediately after removal the tissue was placed in a sterile container and transported to the laboratory on ice. Segments from the mid-portion of each cervix were immersion-fixed in a Hapes-buffered solution containing 3% paraformaldehyde/0.5% glutaraldehyde (Verhage et al., 1979). Samples were fixed overnight at 4°C. Cervix segments were dehydrated in graded ethanol changes and embedded in Araldite. Thick sections (0.5 \(\mu\)m) were cut on a Sorvall MT-5000 ultramicrotome, placed on slides, stained with toluidine blue and photographed.

Explant cultures. Strips of cervical mucosa were cut into 2-3 mm pieces and rinsed: 75 mg tissue were placed in preweighed, 35-mm Petri dishes with 3 ml medium (Fazleabas & Verhage, 1986, 1987) containing 25 \(\mu\)Ci L-\(^{35}\)Smethionine. The tissue and medium were incubated for 24 h at 37°C in a gas-tight chamber (Bellco Biological Glassware, Vineland, NJ, USA) under an atmosphere of 5% CO\(_2\)-40% O\(_2\)-55% N\(_2\) (by volume) on a rocking platform (6 cycles/min).

One and two-dimensional polyacrylamide gel electrophoresis (1- and 2-D PAGE) and fluorography. Gel electrophoresis was carried out according to the procedures described by Roberts et al. (1984) and as utilized for analysis of baboon tissue by Fazleabas & Verhage (1986, 1987).

Immunoblotting of electrophoretically transferred culture media proteins. The protein-blotting technique of Towbin et al. (1979) was used in conjunction with the Hoeffer Trans-Blot system (Hoeffer Scientific, San Francisco, CA,
USA). Culture media proteins (50 μg) from each stage of the cycle were separated on 1-D gels and then immediately transferred to nitrocellulose membranes (Bio-Rad) at 200 mA constant current for 12 h. Following electrophoresis, binding of polyclonal antibodies to human placental proteins (Behringwerke AG, Marburg, FRG) and a monoclonal antibody (mAb) to human pregnancy-associated endometrial secretory α1-globulin (α1-PEG) were detected using the Bio-Rad Immuno-Blot kit according to the manufacturers' specifications. All polyclonal antibodies were diluted 1:500 and the ascites-derived α1-PEG mAb was diluted 1:10 000 and incubations with primary antiserum were carried out for 2 h at room temperature.

Results

Steroid concentrations

These are summarized in Fig. 1 since they are presented in greater detail elsewhere (Fazleabas et al., 1988). The amounts of steroids produced by the implants used in this study were physiological for the baboon when compared with the concentrations present in cyclic animals as measured by us (Fazleabas et al., 1988) and others (Kling & Westfahl, 1978).

Morphology

The mucous membrane of the primate cervix, or endocervix, forms complex furrows or compound clefts called the plicae palmatae (Weiss, 1983). These clefts run in longitudinal, transverse, and oblique directions and may penetrate the entire thickness of the mucosa. The lining epithelium of the plicae palmatae consists of a single layer of steroid-responsive cells. The histological specimens were obtained from the middle region of the endocervix.

In long-term ovariectomized animals the clefts are poorly developed (Fig. 2a) and the lining epithelium consists of a single layer of cuboidal cells (Figs 3a, b). Secretory activity appears to be minimal.

![Fig. 2. The endocervix of the baboon: (a) ovariectomized animal; (b) 14-day oestradiol-treated animal; (c) animal primed with oestradiol and then treated for 14 days with oestradiol and progesterone. Paraffin-wax embedded, × 70.](https://example.com)

The administration of oestradiol for 7 or 14 days to ovariectomized baboons resulted in the development of complex clefts (Fig. 2b) and caused the epithelium to differentiate into tall columnar cells of two principal types, ciliated and secretory cells (Figs 3c, d). The ciliated cells appeared to be more numerous in the epithelium lining the lumen of the cervix (Fig. 3c) than in the epithelium lining the clefts. The ciliated cells were basophilic, contained a centrally placed nucleus and secretory granules appeared to be absent. The secretory cells were basophilic and appeared to
Fig. 3. Morphology of the epithelium lining the lumen (a, c, e) and clefts (b, d, f) of the endocervix of the baboon: (a, b) ovariectomized animal; (c, d) 14-day oestradiol-treated animal; (e, f) animal primed with oestradiol then treated for 14 days with oestradiol and progesterone. Secretory cells are present in the oestradiol-treated animal and the apical tips of these cells fill with heterogeneous secretory granules (d, arrowheads). Secretory activity was greatly reduced in the oestradiol-primed, oestradiol + progesterone-treated animal and some cells were intensely basophilic (e, arrowhead). cc, ciliated cells. Plastic embedded, × 800.
be tallest in the epithelium lining the clefts (Fig. 3d). They were characterized by a basally placed nucleus and a supranuclear cytoplasm completely filled with heterogeneous secretory granules. Mucus was present in the clefts and lumen.

The administration of progesterone for 14 days in the presence or absence of oestradiol to oestrogen-primed animals altered the appearance of the lining epithelium in a manner suggesting dedifferentiation. The clefts were still complex, but they did not appear to be distended as in the oestradiol-treated animals (Fig. 2c). The epithelium was lower and devoid of ciliated cells. Scattered epithelial cells in the luminal epithelium were intensely basophilic (Fig. 3e), suggestive of dying cells. The epithelial cells lining the lumen of the cervix were virtually devoid of secretory activity and secretory apical blebs, whereas many of those lining the clefts still contained apical heterogeneous secretory granules (Fig. 3f).

**Synthesis and release of cervical polypeptides in culture**

Figure 4 illustrates the changes in protein synthesis during the menstrual cycle. Several polypeptides appeared to be synthesized throughout the cycle. Two radiolabelled bands (Mr 160 000 and 23 000) were only present during the late follicular and mid-luteal stages of the cycle while other radiolabelled polypeptides showed some cyclic variation. The administration of oestradiol for 7 or 14 days to ovariectomized baboons dramatically altered the synthetic profile. A group of low molecular weight proteins (Mr 20 000–23 000, Proteins 7–9, Fig. 5f), not present on fluorographs from ovariectomized animals (Fig. 5e), became apparent. These proteins were also present on the 2-D PAGE fluorographs obtained from material at the late follicular stage and
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were from the early luteal animal (Fig. 5c), thus confirming the changes observed on the 1-D PAGE fluorographs (Fig. 4).

Several polypeptides continued to be synthesized during culture by the endocervix obtained from the ovariectomized baboons. The three most intensely labelled proteins (Proteins 1–3, Fig. 5e) were present on all fluorographs included in this study. The fluorograph from the intact baboon at the early follicular stage while very similar to that from the ovariectomized baboons, did contain 3 additional proteins (Proteins 4–6, Fig. 5a) which were also observed on all other fluorographs except those from the ovariectomized animals. The intensity of Proteins 7 and 8 seen predominantly in oestradiol-treated animals was greatly reduced on the fluorographs from the 7-day progesterone-treated (Fig. 5g) and the early luteal (Fig. 5c) animals, whereas Protein 9 remained intense.

The synthetic pattern observed on the fluorographs obtained from the late luteal and oestradiol-primed, 14-day progesterone (+ oestradiol)-treated animals was remarkably similar (Figs 5d and 5h). Proteins 7–9 were totally absent and the high molecular weight basic proteins were greatly reduced in intensity. This synthetic pattern was also very similar to that observed in the early follicular animal and, except for Proteins 4, 5 and 6, in the ovariectomized animal.

Immunological cross-reaction with antibodies to human 'placental' proteins

Because some of the cervical secretory polypeptides migrated in a manner similar to some endometrial products during 2-D PAGE, and because baboon endometrial secretory proteins have been shown to cross-react immunologically with antibodies to some human 'placental' proteins (Fazleabas & Verhage, 1987), we tested endocervical secretions for immunological cross-reactivity with antibodies to human placental proteins (PP). Cultures from intact baboons and all treatment groups were tested against polyclonal antibodies to PP4, PP7, PP12, PP14, PP16, and a monoclonal antibody to α1-PEG which is analogous to PP12 (Bell & Bohn, 1986) after transfer to nitrocellulose membranes. All intact, ovariectomized, and steroid-treated ovariectomized cultures reacted positively with antibodies to PP4 and PP7. No cross-reactivity with the other antibodies could be detected. Figure 6 shows the Western blots of culture medium from intact animals when reacted with the antibodies.

Discussion

This study clearly demonstrates that the ovarian steroids regulate the morphological appearance of the epithelial cells of the baboon endocervix. In the ovariectomized animal the clefts are poorly developed and lined with a cuboidal epithelium. In the oestradiol-treated animal the clefts are well

Fig. 5. Fluorographs of dried 2-D PAGE (10% acrylamide) of aliquants of endocervix culture media (100 000 c.p.m.) labelled with 1-[35S]methionine. Exposure time was 10 days. (a) Early follicular phase. (b) Late follicular phase. (c) Mid-luteal phase. (d) Late luteal phase. (e) Ovariectomized animal. (f) Oestradiol-treated animal. (g) Baboon primed with oestradiol and treated for 7 days with oestradiol + progesterone. (h) Oestradiol-primed animal treated with progesterone for 14 days. Proteins 1–6 (a) were present on all fluorographs except those from ovariectomized animals (e). Proteins 7–9 (b) appeared to be oestradiol-dependent since they were absent on fluorographs from ovariectomized (e) and long-term progesterone-treated animals (d, h).
developed and lined by a tall columnar secretory epithelium. Simultaneous treatment with oestradiol and progesterone in the oestradiol-primed animal resulted in a modest degree of dedifferentiation. Some clefts were still well developed, but the lining epithelium appeared to be low columnar and less secretory.

The morphological changes observed in the treatment groups included in this study correlate well with the polypeptide synthetic pattern. The synthesis of new groups of proteins ($M_\text{r}$ 160 000 and 20 000–23 000) occurred in the oestradiol-dominated animal when compared to untreated ovariectomized animals. The addition of progesterone to the treatment regimen did not appear to result in the synthesis of major new polypeptides, and long-term progesterone appeared to inhibit the synthesis of the two groups of proteins observed in the oestradiol-dominated animals.

Previously we reported that the ovarian steroids also regulate the synthetic activity of the baboon oviduct (Verhage & Fazleabas, 1988) and uterus (Fazleabas & Verhage, 1987; Fazleabas et al., 1988). The fluorographs presented in Fig. 7 illustrate the major synthetic patterns observed in the three reproductive tissue compartments of the baboon. Oestradiol induced the synthesis of new proteins in all three tissues (Figs 5a, c and e, arrows) since they were not present on fluorographs obtained from untreated ovariectomized animals. The addition of progesterone to the treatment regimen prevented the synthesis of the oestradiol-dependent proteins in all three tissues and new proteins were only observed in the uterus (Fig. 5d, arrows). Apparently, progesterone is primarily antagonistic towards the action of oestradiol in the oviduct (Verhage & Fazleabas, 1988) and cervix but synergistic with oestradiol in the uterus (Fazleabas et al., 1988). These synthetic patterns are directly correlated with the reproductive function of these three tissues. The oviduct and cervix are responsible for gamete transport at or near the time of ovulation, a period of oestradiol dominance, while the endometrium supports the embryo during the luteal phase and pregnancy, a period of progesterone dominance.

Presumably, the steroid-dependent proteins identified in the oviduct, uterus and endocervix are also tissue-specific since they differ in molecular weight and pI. This is a reasonable assumption since antibodies to the $M_\text{r}$ 120 000 glycoproteins of the oviduct do not cross-react with the secretory products of the baboon endometrium and endocervix (Verhage et al., 1989). However, Fig. 7 also illustrates that some polypeptides synthesized by the endocervix are electrophoretically similar to those synthesized by the oviduct and uterus (Fig. 7, Proteins 1–3). Others appear to be limited to the endometrium and endocervix (Fig. 5, Proteins 4–6). The synthesis of these more ubiquitous proteins does not appear to be regulated by the ovarian steroids.

Media from endocervical cultures were also tested for the presence of 'placental' proteins (PP). PP$_4$ (anticoagulant protein; Grundmann et al., 1988) and PP$_7$ (glutathione S-transferase enzyme; Bohn, 1985) are readily identifiable on endocervical immunoblots. These two polypeptides, the
Fig. 7. Fluorographs of 2-D PAGE comparing the major protein patterns of the oviduct (a, b; 7.5% acrylamide); uterus (c, d; 10% acrylamide) and cervix (e, f; 10% acrylamide) observed in ovariectomized baboons treated with oestradiol treatment for 14 days (a, c, e) or primed with oestradiol and treated with oestradiol + progesterone for 14 days. The major steroid-dependent macromolecules are indicated by arrowheads for each tissue.

Functional significance of which in reproduction has yet to be defined, are ubiquitous components of the female baboon reproductive tract, and their synthesis does not appear to be regulated by the ovarian steroids (Fazleabas & Verhage, 1987; Verhage et al., 1988). The absence of any of the other
immunoreactive PPs in endocervical culture media contrasts with our findings in endometrial cultures (Fazleabas & Verhage, 1987). Of particular interest was the absence of an immunoreactive product which cross-reacted with a polyclonal antibody to PP$_{12}$ or a monoclonal antibody to its analogous molecule, a$_1$-PEG (Bell & Bohn, 1986). This protein is the insulin-like growth factor binding protein identified in human endometrium and decidua (Bell et al., 1988) and has also been shown to be identical to a protein synthesized by baboon tissues (Fazleabas et al., 1989).

The oviduct, uterus and cervix have a common embryological origin. However, our results clearly show, morphologically and biochemically, that each segment of the reproductive tract responds to the ovarian steroids in a very specific manner and that this response appears to be correlated with reproductive function. We are attempting to purify the steroid-dependent polypeptides identified in each tissue, and develop antibodies towards them. These studies should provide additional insight into the functional significance of these proteins in each of the individual compartments of the female reproductive tract.

These studies were supported by National Institute of Health grants HD21991 and HD20571. We thank Ms Kathy Donnelly and Ms. Patty Mavrogianis for excellent technical assistance; Dr Stephen C. Bell, University of Leicester, for his kind gift of a$_1$-PEG monoclonal antibody; and Ms Margarita Guerrero for typing this manuscript.

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Received 5 October 1988