Cyclic Changes in Epidermal Growth Factor Receptor in Human Endometrium during Menstrual Cycle

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Abstract

Epidermal growth factor (EGF), a potent mitogenic peptide, is known to be present in the fluid of the uterine cavity. Recent studies have demonstrated the messenger RNA for EGF in the rat uterus. Therefore, in an attempt to clarify its physiological role, we investigated the receptors for EGF in human endometrial tissues. The particulate fractions from endometrium possessed the capacity to bind EGF in a specific, saturable and reversible manner. The Scatchard plot was linear, showing a single class of the receptor with an apparent Kd of $3.8 \times 10^{-9}$ M. The amount of specific EGF binding was very low during menstruation and increased gradually, reaching its peak in the late follicular phase. There was an abrupt decline in the binding after ovulation with no change in the Kd value. These results imply the possible involvement of EGF in the process of proliferation of human endometrial tissues.

Epidermal growth factor (EGF) is a single-chain polypeptide of 6045 daltons, first isolated by Cohen (Cohen et al., 1962) from submandibular glands of male mice. EGF binds to specific cell surface receptors to elicit diverse biological actions both in vivo and in vitro, including cell proliferation and regulation of differentiated cellular functions.

In recent studies, it was reported that EGF is present in the uterine luminal fluid in the rat (Imai 1982) and in the mouse uterus. (Gonzalez et al., 1984). Moreover, the presence of EGF receptors was demonstrated in the rat and in the human myometrium (Hofmamm et al., 1984). In view of these findings, it is of great interest to investigate the receptors for EGF in the human endometrium at different stages in the menstrual cycle, since the functional state of endometrial cells undergoes drastic changes during the menstrual cycle.

Here we report the presence of EGF receptors in human endometrium and its cyclic variation during the menstrual cycle.

Materials and Methods

Materials

Endometrial tissues were obtained from women undergoing hysterectomy for reasons other than endometrial pathology. The women aged 38-45 years with a history of regular menstrual cycles had not been taking any hormone therapy for at least 2 cycles before
operation. Informed consent was obtained from each one. The tissue was dated by histological examination. Endometrial tissues were stored at -80°C until required for assay.

Mouse EGF (receptor grade), fibroblast growth factor, multiplication stimulating activity, nerve growth factor and platelet-derived growth factor were purchased from Collaborative Research.

Tissue homogenate

The tissues were homogenized at 4°C in 10 volumes of 50 mM Tris-HCl buffer, PH 7.4 containing 10 mM MgCl₂ in a polytron homogenizer with the dial set at 7 for 20 sec, followed by centrifugation at 12000 g for 20 min. The resultant precipitates were resuspended in an equal volume of the homogenizing buffer.

Iodination of EGF

Iodination of EGF was performed by the chloramine-T technique as described. (Hunter et al., 1962). The labeled hormone was separated from unreactive Na¹²³I by passing through a Sephadex G-50 column. Iodinated EGF had a specific activity of about 200 µCi/µg.

¹²⁵I-EGF binding assay

¹²⁵I-EGF (about 120,000 cpm) was incubated with various concentrations of unlabeled EGF (0–100 ng/tube) and 100 µl of homogenate protein in a final volume of 500 µl buffer consisting of 50 mM Tris-HCl buffer, PH 7.4 containing 10 mM MgCl₂ and 0.1% bovine serum albumin. After incubation, 2 ml of the same ice-cold buffer was added into each tube and the tubes were centrifuged at 2,000 g for 20 min at 4°C. The radioactivity of the pellets was counted in a gamma counter. Specific ¹²⁵I-EGF binding was determined by subtracting the amount of radioactivity bound nonspecifically in the presence of 1 µg unlabeled hormone.

Results

The ¹²⁵I-EGF binding was linear with the homogenate protein ranging from 125 to 1000 µg/0.5 ml (data not shown). In these studies, the protein concentrations were adjusted to approximately 300 µg/0.5 ml.

Fig. 1 shows the time course of EGF binding to human endometrial tissues at different temperatures. Apparent equilibrium was reached at 30 min at 37°C, whereas the binding increased gradually up to 120 min at 24°C and leveled off thereafter. From
these data, the EGF binding was shown to be a saturable process. Subsequent binding studies were carried out for 120 min at 24°C.

The binding of labeled EGF to endometrial tissues was specific in the sense that the unlabeled EGF at 32 ng/ml caused about 50% suppression of binding as shown in Fig. 2, while unlabeled fibroblast growth factor (FGF), multiplication stimulating activity (MSA), nerve growth factor (NGF) (each at 1 μg/ml), platelet-derived growth factor (PDGF) at 100 ng/ml and porcine insulin at 5 μg/ml do not compete with

Fig. 2. Competition of unlabeled EGF with 125I-EGF for binding to human endometrial tissues. Endometrial tissues were incubated with 125I-EGF and different concentrations of unlabeled EGF. Specific binding was expressed as the percentage of that occurring in the absence of unlabeled EGF. Each value represents the mean of triplicate determinations.

![Graph showing competition of unlabeled EGF with 125I-EGF for binding to human endometrial tissue.](image)

Dissociation of 125I-EGF from Human Endometrial Tissue with Time

![Graph showing dissociation of 125I-EGF from human endometrial tissue.](image)

Fig. 3. Dissociation of 125I-EGF binding from human endometrial tissues with or without EGF. Particulate fractions of endometrium were equilibrated with 125I-EGF for 2 hours at 24°C. The pellet was resuspended in the same buffer with or without EGF (1 μg/ml) and dissociation of bound radioactivity was evaluated at the times indicated. Remaining 125I-EGF at a given time is expressed as a percentage of the initial bound radioactivity.
iodinated EGF binding.

Fig. 3 illustrates the dissociation curves for EGF binding. Although the dissociation rate was higher when the receptors were saturated with excess unlabeled EGF, less than 50% of the initial binding remained at 30 min in the absence of EGF.

The specific binding of EGF to endometrial tissues at different stages of menstruation is shown in Fig. 4. The amount of specific binding increased from the early proliferative to the late proliferative phase at different stages of menstruation.

**Fig. 4.** Changes in the specific binding of $^{125}$I-EGF to human endometrial tissues during the menstrual cycle. $^{125}$I-EGF binding was conducted as described under "Materials and Methods". The menstrual cycle was determined by BBT records and further verified by histological examination.

**Fig. 5.** Scatchard plot of $^{125}$I-EGF binding to human endometrial tissues in the late follicular and the late secretory phase.
and leveled off until the early secretory phase. Four to 6 days after ovulation, a precipitous decline in EGF binding occurred and the amount of binding remained low up to menstruation.

Fig. 5 shows the Scatchard plot of $^{125}$I-EGF binding in the late proliferative and late secretory phase. Both Scatchard plots were linear, showing a single class of receptor for EGF (kd: $3.8 \times 10^{-9}$ M). However, the number of receptors in the late proliferative phase was about four times as great as in the late secretory phase.

Discussion

In an earlier work, the presence of EGF receptor in cultured human uterine smooth muscle cells was documented (Bhargava et al., 1979). Recent studies also have demonstrated the specific receptors for EGF in myometrium, leiomyoma and endometrium in human (Hofmann et al., 1984). However, no information is yet available about the number of EGF receptors with respect to the menstrual cycle. In the present series of studies, we have shown that the amount of EGF binding to human endometrial tissues is altered during the menstrual cycle.

Our results observed a linear Scatchard plot indicative of a single class of receptor for EGF with a Kd value of $3.8 \times 10^{-9}$ M. Sheets et al., also reported the EGF receptor in human endometrial tissues with a Kd value of $3 \times 10^{-9}$ M, in good keeping with results of the present study (Sheets et al. 1985). The nanomolar range of Kd values was also demonstrated in human endometrium by Hofmann and in human liver by Lev-Ran (Lev-Ran et al., 1984). The Kd value in the rat uterus was $3.6 \times 10^{-10}$ M (Mukku et al., 1985) implying a difference between species. However, it is not pertinent to compare the Kd value for the rat with that for man since the Kd value for the rat represents the value utilizing the whole uterus without separating the endometrial tissues as in human.

The present study emphasizes the fluctuation in the amount of the EGF binding in human endometrium during the menstrual cycle. However, Sheets et al. found no difference between EGF binding in the proliferative and secretory phases. A likely explanation for this is that the specimens obtained were divided into two groups, i.e. proliferative or secretory phase in their study. As shown in this study, the amount of EGF binding undergoes drastic changes during each phase. Hence, if the specimens from each phase are put together, the changes in EGF binding could not be detected.

No doubt estrogen plays a pivotal role in the proliferation of the endometrium, but the mechanism whereby estrogen influences endometrial cells is still poorly understood. Recently, it was suggested that certain growth factors mediate estrogen action in inducing cell division in estrogen stimulated target cells. For instance, insulin-like growth factor or EGF-like peptides were detected in the conditioned medium of human breast cancer cells cultured with estrogen (Lippman et al., 1984). Furthermore, according to the results of a more recent study, the mouse uterus is shown to contain considerable amounts of EGF, the levels of which are elevated by estrogen (DiAugustine et al., 1985). Taking the results of these observations together, it is tempting to speculate that EGF may be involved in estrogen stimulated uterine growth.

Mukku et al. have reported that the administration of estradiol to immature female rats produces a 3-fold increase in EGF binding to uterine membrane with no change in the affinity of membrane receptors for EGF (Mukku et al., 1985). It was also shown that the effect of estradiol is specific in that other steroid hormones fail to increase the number of the receptors. These
findings lead us to suggest that ovarian sex steroids may take part in the alteration of EGF receptors in the endometrium as shown here. Preliminary studies of a primary cell culture of human endometrium demonstrated the increase in the binding of EGF to endometrial cells cultured in the presence of estradiol. Another example of the regulation of EGF receptors by steroid hormones is the increased ability of human fibroblasts to bind EGF in the presence of dexamethasone (Baker et al., 1978). Progestin is also known to increase EGF receptors in a certain cell line of human breast cancer (Murphy et al., 1985).

The induction of EGF receptors by estrogen occurs between 6 and 12 hours after the hormone administration whereas DNA synthesis begins approximately 18–24 hours after the treatment (Mukku et al., 1985). Thus, the increase in EGF receptors precedes the DNA synthesis. Another line of evidence indicates that several events occurring between 6 and 12 hours after the estrogen treatment play a critical role in inducing uterine growth (Anderson et al., 1975, Harris et al., 1978). These findings further suggest a possible role of EGF in mediating a mitogenic action of estrogen.

Gonzalez et al. have revealed that the sequential injection of estradiol to immature mice effects an increase in uterine weight and the concentrations of EGF in the uterus (Gonzales et al., 1984). However, the EGF concentration in the submandibular gland is unaltered by estradiol. The submandibular gland is thought to be a major source of EGF. Recent studies imply that EGF is produced in some tissues other than the submandibular gland, such as the kidney and the pancreas (Rall et al., 1985). More recently, messenger RNA for EGF was detected in the uterus of ovariectomized rats (Teng et al., 1985), and the levels of the messenger RNA are augmented by estrogen treatment in keeping with the findings of Gonzales et al. These facts indicate a possible paracrine or autocrine role of EGF involved in the regulation of uterine growth.

References


