MOLECULAR REPRODUCTION AND DEVELOPMENT 50:63-69 (1998)

Regulation of Plasminogen Activator in Rat Endometrial Stromal Cells: The Role of Epidermal Growth Factor

BRENT M. BANY,[1] XINGQI ZHANG,[2] AND THOMAS G. KENNEDY[1]*

[1]*Departments of Physiology and Obstetrics and Gynaecology, University of Western Ontario, London, Ontario, Canada*
[2]*Department of Obstetrics and Gynaecology, University of Calgary, Calgary, Alberta, Canada*

ABSTRACT The effect of epidermal growth factor (EGF) on the accumulation of plasminogen activator (PA) activity in the medium of cultured rat endometrial stromal cells isolated from uteri sensitized for the decidual cell reaction was examined. Treatment with EGF increased, in a concentration-dependent manner, PA activity in the medium. This effect was inhibited or greatly reduced by inhibitors of transcription and translation. Incubation of the cells with prostaglandin E_2 increased PA activity in the medium. Indomethacin, which inhibited prostaglandin accumulation in the medium, slightly but significantly decreased the EGF-induced increase in PA activity in the medium. As indicated by zymography and the use of amiloride in the PA assay, the activity in the medium was primarily urokinase-type plasminogen activator (uPA). Finally, EGF caused an increase in the steady-state uPA mRNA levels in the cells. These results provide evidence that EGF causes an increase in the secretion of uPA by rat endometrial stromal cells from uteri sensitized for the decidual cell reaction through a mechanism that involves an increase in steady-state uPA mRNA levels. Mol. Reprod. Dev. 50:63-69, 1998.

Key Words: epidermal growth factor; plasminogen activator; urokinase-type plasminogen activator; endometrial stromal cells; uterus

INTRODUCTION

Decidualization is the process by which the fibroblast-like endometrial stromal cells differentiate into large decidual cells that eventually form the maternal component of the placenta. In rodents, decidualization begins in the endometrium in response to an implanting blastocyst or artificial stimuli (Psychoyos, 1973). It will only be initiated over a specific period of time during pregnancy, pseudopregnancy, or in ovariectomized animals when the uterus has been sensitized by an appropriate regimen of hormones (Finn and Porter, 1975; Psychoyos, 1973). Rat endometrial stromal cells also will decidualize in vitro if they are isolated from uteri sensitized for decidualization (Vladimirsky et al., 1977). This culture system provides a good model for studying the regulation of decidualization.

Decidualization of the endometrium involves substantial tissue remodeling (Finn, 1986; Weitlauf, 1994). Since plasminogen activators (PAs) are believed to play a role in tissue remodeling in other tissues (Danø et al., 1985), they may have a role in endometrial remodeling during decidualization. There are two forms of PAs, the tissue-type (tPA) and urokinase-type (uPA) (Danø et al., 1985). The secretion of uPA, but not tPA, by rat endometrial stromal cells dramatically increases during in vitro decidualization (Zhang et al., 1996). Further, in vivo, uPA is expressed in endometrial stromal cells undergoing decidualization during early pregnancy and after artificially induced decidualization (Wang et al., 1996). Therefore, it appears that uPA expression increases in rat endometrial stromal cells during decidualization in vivo and in vitro. However, little is known about the regulation of uPA gene expression during implantation and decidualization.

Epidermal growth factor receptor (EGFR) and some of its ligands are present in the rodent uterus during implantation (Das et al., 1994a, 1994b; Johnson and Chatterjee, 1993; Paria et al., 1994; Tamada et al., 1991; Tong et al., 1996). Although they are present in the uterus, the function of the EGFR and its ligands in implantation is not clear. However, one possible role of ligands of the EGFR may be to modulate uPA secretion by endometrial stromal cells. In support of this possible role is the observation that epidermal growth factor (EGF) stimulates the secretion of uPA by human endometrial stromal cells (Miyauchi et al., 1995) and by several other nonuterine cell types (Jensen and Rodeck, 1993; Tranque et al., 1994). Further, EGF stimulates prostaglandin (PG) accumulation (Bany and Kennedy, 1995), and PGs stimulate uPA secretion by cultured rat endometrial stromal cells (Zhang et al., 1996). There-

X. Zhang's present address: Department of Obstetrics & Gynecology, Reproductive Endocrinology and Infertility Section, Northwestern University Medical School, Chicago, IL.

*Correspondence to: Dr. T.G. Kennedy, Department of Physiology, The University of Western Ontario, M223 Medical Sciences Building, London, Ontario N6A 5C1, Canada.
E-mail: tkennedy@physiology.uwo.ca

Received 13 August 1997; Accepted 11 November 1997

fore, the objectives of the present study were to determine the effect of **EGF** on uPA secretion and steady-state mRNA levels in rat endometrial stromal cells isolated from uteri sensitized for decidualization.

MATERIALS AND METHODS

Animals

Female Harlan Sprague-Dawley rats (200-225 g) (Harlan Sprague-Dawley, Inc., Indianapolis, IN) were housed under temperature- and light-controlled conditions (lights on from 0500 to 1900 hr) with free access to food and water. The animals were ovariectomized under ether anesthesia and allowed at least 5 days to recover. To obtain rats with uteri sensitized for decidualization, estradiol and progesterone (Sigma Chemical Company, St. Louis, MO) in sesame oil were administered subcutaneously as described elsewhere (Bany and Kennedy, 1995).

Endometrial Stromal Cell Isolation

Endometrial stromal cells were obtained from the sensitized uteri as described elsewhere (Bany and Kennedy, 1995; Yee and Kennedy, 1991). The stromal cells were suspended in Dulbecco's Modified Eagle's Medium: F-12 nutrient mixture (DMEM:F-12) containing penicillin **(50** units/ml), streptomycin (50 µg/ml), fungizone (1.25 µg/ml), and 10% heat-inactivated charcoal-stripped fetal calf serum (all from Gibco-BRL, Burlington, Ontario, Canada). The cell suspension was filtered through nylon mesh (70 pm) to remove glands and clumps of cells. The cells were plated at 5 X 10^5 cells (in 0.5 ml of medium) in 24-well plates (Becton-Dickinson, Lincoln Park, NJ) and incubated at 37°C under 5% CO_2 and 95% air for 2 hr to allow for differential attachment of the stromal cells, after which the medium and free-floating cells were removed and replaced with serum-free DMEM:F-12 containing antibiotics and fungizone. This was defined as 0 hr of day 1 of culture. The resulting cultures of attached cells were free of contaminating epithelial-type cells, as indicated by the absence of positively staining cells for cytokeratin by immunocytochemistry (data not shown).

Cells were incubated with or without various treatments for 24 hr on day 3 of culture in serum-free medium. Treatments included human recombinant EGF (Gibco-BRL), cycloheximide, actinomycin D, indomethacin (IM) (Sigma), and prostaglandin E_2 (PGE_2) (Cayman Chemical Company, Ann Arbor, MI). Cycloheximide, prostaglandin E_2, and IM were dissolved initially in ethanol and were added to the culture medium so that the amount of ethanol did not exceed 0.5%; in these experiments, cells not receiving these treatments were cultured with the same concentration of ethanol. After treatment for 24 hr, the medium was collected, stored at -20°C, and thawed just prior to determination of PA activity or PGE_2 levels. To determine the amount of cellular protein in each well, the cells were washed with Dulbecco's phosphate-buffered saline (Gibco-BRL) after the medium was removed, solubilized in 1% SDS-l mM EDTA, and assayed by the method of Lowry et al. (1951) using bovine serum albumin (Sigma) as the standard.

Plasminogen Activator Assays and PGE_2 Radioimmunoassay

Net PA activity in the medium was measured using the chromogenic assay of Coleman and Green (1981), as modified by Zhang et al. (1996). In brief, samples were incubated with 25 ng of plasminogen (Sigma) in 96-well microtiter plates for 30 min to generate plasmin. Chromogenic substrates for plasmin, 5,5'-dithio-*bis*(2-nitrobenzoic acid) (0.22 mM) and N-a-carbobenzoxy-L-lysine thiobenzyl ester (0.18 mM) (Sigma), were then added, and after 15-30 min the absorbance at 405 nm was determined using an automated microplate reader. Standard curves were constructed using 0-20 mIU of human urokinase (Calbiochem, San Diego, CA). Net PA activity in the medium was expressed as International Units per milligram of cellular protein. To determine the nature of the PA activity in the samples, amiloride (200 µM; Sigma) (a specific inhibitor of uPA; Vassalli and Belin, 1987) was added to some assays. In addition, the PA activity was further characterized using a modified enzymography assay (Heussen and Dowdle, 1980), as described previously (Zhang et al., 1996). **PA** activity was revealed as a cleared zone (band) in the zymography gel. Relative molecular masses of these bands were determined using prestained molecular mass markers (Amersham, Oakville, Ontario, Canada).

PGE_2 was measured directly in the medium by radioimmunoassay, as described previously (Bany and Kennedy, 1995). PGE_2 accumulation was expressed as nanograms of PGE_2 per milligram of cell protein.

Northern Blot Preparation and Hybridization

Total ribonucleic acid (RNA) was isolated by a single-step guanidinium thiocyanate method (Chomczynski and Sacchi, 1987). The RNA samples (10 µg per lane) were denatured in MOPS buffer (10 mM MOPS, 5 mM sodium acetate, and 1 mM EDTA, pH 7.2) containing 6% formaldehyde and 50% formamide at 65°C for 15 min. They were then subjected to denaturing agarose gel (1.1%) electrophoresis and transferred to Hybond-N membranes (Amersham) by capillary transfer, as described by Sambrook et al. (1989). The RNA was cross-linked to the membranes by ultraviolet radiation (0.12 J/cm^2).

The membranes were prehybridized for 3 hr in buffer containing 1 M NaCl, 50 mM Tris, 2.2 mM sodium pyrophosphate, 1% SDS, and 10X Denhardt's reagent (Sambrook et al., 1989), pH 7.5, at 65°C for 2 hr. The cDNA probe for uPA has been described previously (Zhang et al., 1996). The uPA probe (20 ng) was labeled by the random priming technique in the presence of ^{32}P-dCTP (Amersham) using a Klenow fragment kit obtained from ID Labs (London, Ontario, Canada). Hybridization was carried out at 65°C for 18 hr, followed by two washes with 2X SSC (0.03 M sodium citrate, 0.3 M NaCl, pH 7)-l% SDS and two washes in 0.2X SSC-1% SDS at 65°C for 15 min. The membranes

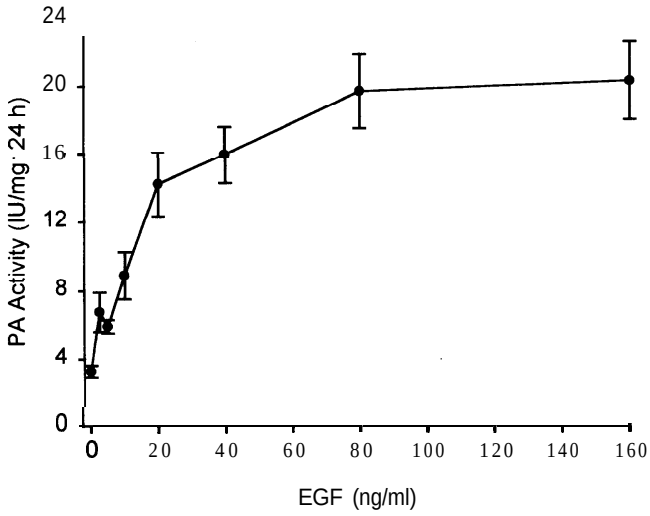

Fig. 1. Concentration-response relationship between epidermal growth factor (EGF) and net plasminogen activator (PA) activity. Cultured rat endometrial stromal cells were treated with EGF for 24 hr on day 3, and net PA activity in the medium was measured. Each point represents the mean ± SEM (n = 6).

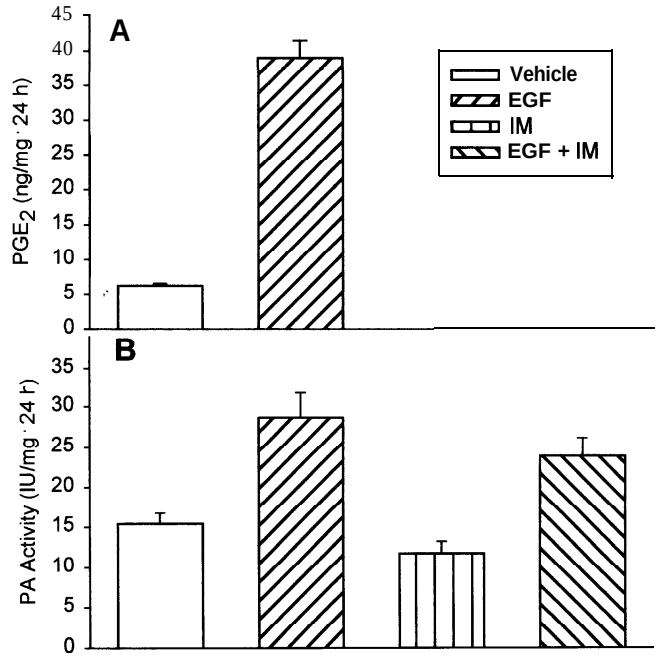

Fig. 2. Effects of indomethacin (IM, 10^{-5} M) and epidermal growth factor (EGF, 40 ng/ml) on prostaglandin E_2 (PGE_2) accumulation and net plasminogen activator (PA) activity. Cultured rat endometrial stromal cells were treated with or without IM and EGF for 24 hr on day 3 and **(A)** PGE_2 accumulation and **(B)** net PA activity in the medium were determined. Each bar represents the mean ± SEM (n = 5).

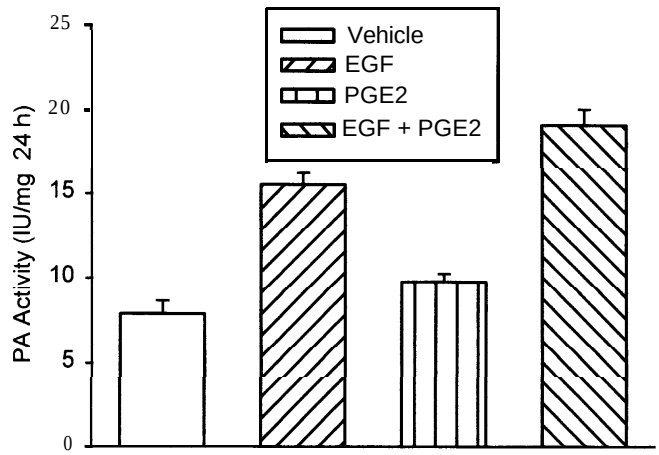

Fig. 3. Effects of prostaglandin E_2 (PGE_2, 1 µg/ml) and epidermal growth factor (EGF, 40 ng/ml) on net plasminogen activator (PA) activity. Cultured rat endometrial stromal cells were treated with or without PGE_2 and EGF for 24 hr on day 3 and PA activity in the medium was determined. Each bar represents the mean ± SEM (n = 6**).**

were then subjected to autoradiography at -70°C with intensifying screens. After autoradiography, membranes were stripped of hybridized probe by immersion in 1 mM Tris, 1 mM EDTA, and 0.1X Denhardt's reagent (pH 8) for 2 hr at 75°C. Next, they were probed with a cDNA for mouse 18S ribosomal RNA (rRNA) (Smith and Hammond, 1991), labeled as above, in order to determine the relative amounts of RNA loaded into each lane, and transferred to the membranes. The relative level of uPA messenger RNA (mRNA) was determined by image analysis of the autoradiographs, and the results were expressed as the ratio of uPA mRNA signals to 18S rRNA signals.

Statistical Analysis

Heterogeneity of variance was removed by logarithmically transforming all data before analysis. Analysis of variance was used to determine treatment effects. When significant interactions ($P < 0.05$) were detected, Duncan's multiple-range test was used for group comparisons. All statistical analyses were carried out using SAS statistical software (Cary, NC).

The data are presented as means ± SEM (n = 4-6) from single experiments. Experiments were performed two to four times on different endometrial stromal cell preparations. Because of significant differences between experiments, as determined by analysis of variance, the data from different experiments were not pooled. In all experiments, the treatment effects were qualitatively, although not always quantitatively, consistent.

RESULTS

In the first series of experiments, repeated three times with different stromal cell isolations, the effect of incubating cells with various concentrations of EGF (2.5-160 ng/ml) for 24 hr on day 3 of culture on net PA activity in the medium was determined (Fig. 1). Compared with vehicle-treated cells, PA activity in the medium was significantly ($P < 0.01$) increased at all concentrations of EGF used. PA activity in the medium was not significantly different ($P > 0.05$) between 40, 80, and 160 ng/ml EGF. At this maximal stimulation (40

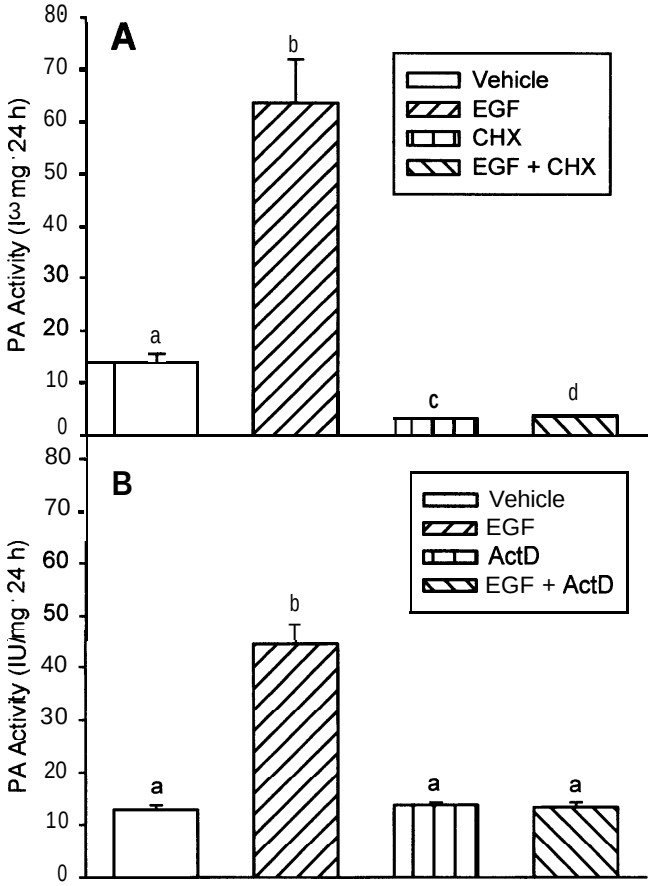

Fig. 4. Effects of inhibitors of transcription and translation on epidermal growth factor (EGF)-induced plasminogen activator (PA) activity in the medium of rat endometrial stromal cells. Cells were incubated with or without **(A)** cycloheximide (CHX, 0.5 µg/ml) or **(B)** actinomycin D (ActD, 1 µg/ml) in the presence or absence of EGF (40 ng/ml) for 24 hr on day 3 of culture. Each bar represents the mean ± SEM (n = 6). Bars with different letters are significantly ($P < 0.05$) different.

TABLE 1. Effect of Amiloride (200 µM) on Net Plasminogen Activator (PA) Activity (IU/ml) in Samples of Medium Obtained From Cells Treated With or Without EGF (40 ng/ml) for 24 hr on Day 3 of Culture

	Treatment	
Assay conditions	Control	EGF
No amiloride	1.24 + 0.10	3.21 ± 0.01
Amiloride	0.06 ± 0.01	0.34 ± 0.08

Note: Values represent the mean ± SEM (n = 5) net PA activity.

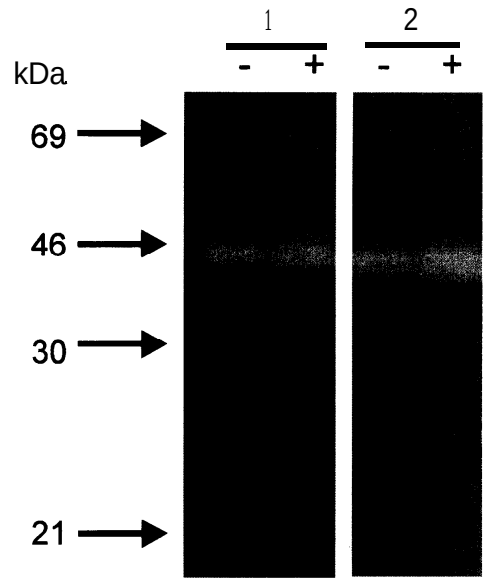

Fig. 5. Zymographic analysis of medium (5 µl) from cells treated without (-) or with (+) epidermal growth factor (40 ng/ml) for 24 hr on day 3 of culture. Representative zymograph of a gel containing samples from two separate experiments (1 and 2). Zones of clearing represent PA-like activity.

ng/ml), EGF caused approximately a 6-fold increase in PA activity.

In the next set of experiments, repeated twice with different stromal cell preparations, IM was used to determine the effect of inhibiting PG production on the EGF-induced increase in net PA activity in the medium. Cells were incubated with or without EGF (40 ng/ml) in the presence or absence of IM (10^{-5} M) on day 3 of culture for 24 hr. EGF caused a significant ($P < 0.001$) increase in PGE_2 accumulation in the medium (Fig. 2A). PGE_2 was not detected (<0.15 ng/ml) in the medium of cells treated with IM or IM plus EGF, verifying inhibition of PG production. In the presence or absence of EGF, IM decreased net PA activity in the medium slightly but significantly ***(P < 0.05; see*** Fig. 2B). In the absence or presence of IM, EGF significantly ($P < 0.001$) increased net PA activity in the medium by 2.4- and 2. l-fold, respectively

The effect of PGE_2 on the EGF-induced increase in net PA activity in the medium was determined in the next series of experiments, repeated four times with different stromal cell isolations. Cells were incubated without or with EGF (40 ng/ml) in the absence or presence of PGE_2 (1 µg/ml) for 24 hr on day 3 of culture (Fig. 3). Both EGF and PGE_2 caused significant ***(P < 0.005)*** increases in net PA activity in the medium; these effects were additive, as indicated by the absence of a significant interaction ***(P > 0.05)*** on analysis of variance.

Cycloheximide (0.5 µg/ml) and actinomycin D (1 µg/ml) were used to determine if the EGF-induced increase in net PA activity required protein synthesis and transcription, respectively (Fig. 4). Cells were incubated without or with EGF (40 ng/ml) in the presence or absence of inhibitor for 24 hr on day 3 of culture in three separate experiments using cells from different stromal cell isolations. For both cycloheximide and actinomycin D, analysis of variance revealed significant interactions ***(P < 0.001)*** between the effects of inhibitor and EGF. The stimulatory effect of EGF on net PA activity was greatly reduced and prevented by

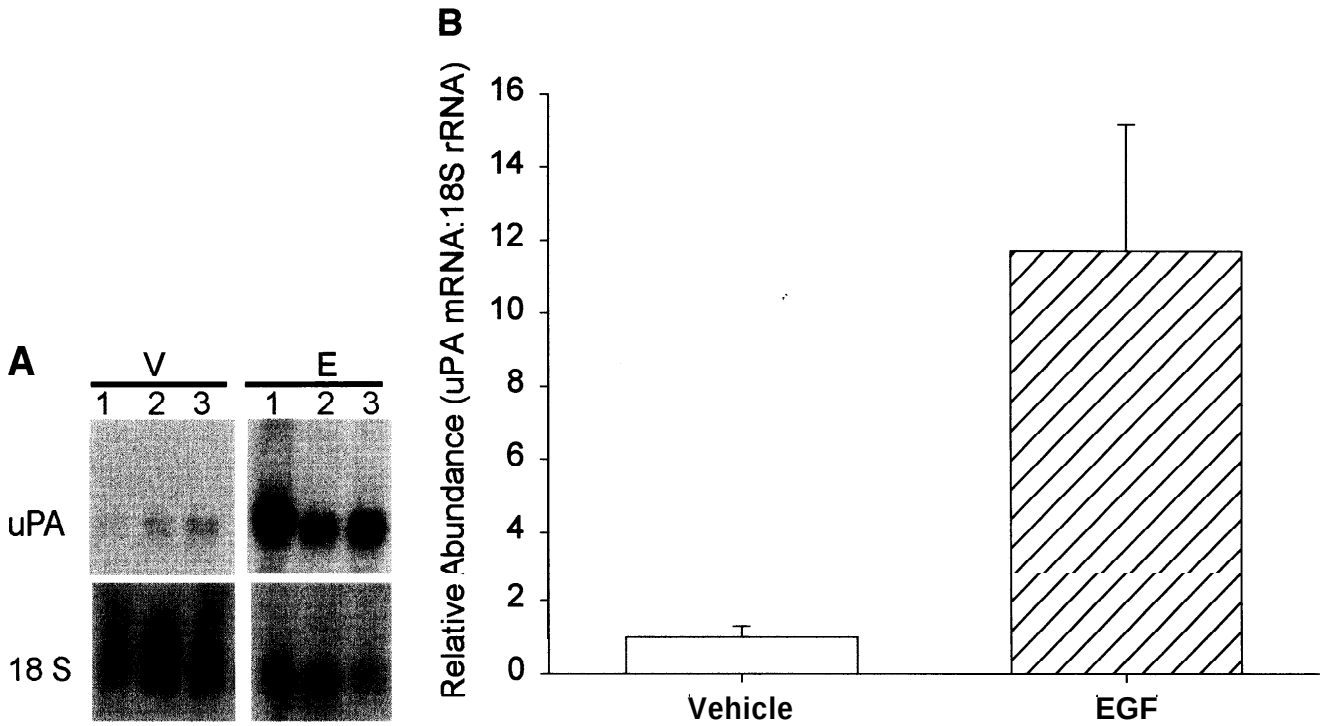

Fig. 6. Northern blot analysis of the effect of epidermal growth factor (EGF) on steady-state uPA mRNA levels in cultured rat endometrial stromal cells. Cells were incubated without (V) or with (E) EGF (40 ng/ml) for 24 hr on day 3 of culture. Panel **A** shows representative autoradiographs of a membrane containing samples from three separate experiments (l-3). Panel **B** shows mean (± SEM; N = 4) ratio of uPA mRNA/18S rRNA signals, as determined by image analysis, with the ratios for the vehicle-treated cells set to 1 for the two groups.

cycloheximide and actinomycin D, respectively. In the presence of cycloheximide, basal net PA activity was significantly reduced ($P < 0.05$) compared with control values.

The nature of PA activity in the media was assessed by the use of amiloride and by zymography. Net PA activity in the conditioned medium from cells incubated without or with EGF (40 ng/ml) was inhibited almost completely when the uPA-specific inhibitor amiloride (200 μM) was added to the chromogenic assay (Table 1). The zymographic analysis detected a single band of clearing corresponding to a protein of approximately 40 kDa (Fig. 5).

Northern blot analyses were used to determine the effect of EGF on the steady-state level of uPA mRNA in the cultured cells. Cells from three independent preparations were incubated with or without EGF (40 ng/ml) for 24 hr on day 3 of culture. A single species of mRNA with a size of approximately 2 kb was detected with the uPA probe (Fig. 6A). The relative level of steady-state uPA mRNA in the cells, as measured by the ratio of the mRNA signals to 18S rRNA signals, was significantly ($P < 0.05$) increased in response to EGF (see Fig. 6B).

DISCUSSION

The present study shows that EGF, in a concentration-dependent manner, increased net PA activity in the medium of cultured rat endometrial cells isolated from uteri sensitized for decidualization. This net PA activity was likely due mainly to uPA, as determined by inhibition by amiloride. Further, zymography revealed a single band of PA activity at approximately 40 kDa that corresponds to the expected molecular mass of uPA and not tPA (Vassalli and Belin, 1987). These combined results strongly suggest that the EGF-induced increase in net PA activity in medium was due mainly to an increase in uPA, and not tPA, activity.

Previous work has indicated that rat endometrial stromal cells secrete uPA in response to PGE_2 (Zhang et al., 1996). Because EGF increases PGE_2 accumulation in the medium of these cells (Bany and Kennedy, 1995), the present observation of an EGF-induced increase in net PA activity may have been mediated by PGE_2. However, IM reduced PGE_2 accumulation in the medium to undetectable levels with only a small (albeit statistically significant) effect on the EGF-induced uPA activity. Further, maximally effective concentrations of EGF and PGE_2 were additive. These results indicate that the increase in uPA activity in the medium in response to EGF cannot be explained entirely by the effect of EGF on PG accumulation in the medium.

The chromogenic assay used in this study measures net PA activity. Therefore, the increase in net uPA activity in response to EGF may have been due to increased uPA secretion and/or decreased secretion of inhibitors of uPA, such as PA inhibitor 1 (PAI-1) and PA inhibitor 2 (PAI-2) (Blasi et al., 1987). In our previous study, PGE_2 increased uPA secretion and its mRNA

levels in cultured rat uterine cells without any effect on PAI-1 (Zhang et al., 1996). Although we did not assess the secretion of uPA inhibitors in this study, we did find that the steady-state level of uPA mRNA increased in response to EGF. In addition, inhibitors of transcription and translation prevented or greatly reduced the effect of EGF on net uPA activity. Therefore, the increase in net uPA activity in response to EGF is likely due, at least in part, to an increase in steady-state mRNA levels of uPA. However, further studies are required to determine the effects of EGF on the expression of inhibitors of uPA in rat endometrial stromal cells.

Several observations suggest that ligands of EGFR may be involved in modulating stromal cell function in the endometrium of rodents during implantation. First, functional EGFR protein (Das et al., 1994b) and mRNA (Tong et al., 1996) are present primarily in the endometrial stroma and not epithelium during implantation in mice. This suggests that any EGFR ligands that are present in the endometrium during implantation target primarily the stromal compartment. Second, during the periimplantation period, the rodent endometrium produces several EGFR ligands, including heparin-binding EGF plus amphiregulin (Das et al., 1995) in the luminal epithelium, and transforming growth factor a in both the epithelium and decidualizing stromal (Paria et al., 1994; Tamada et al., 1991). These ligands may act in an autocrine or paracrine manner on stromal cells. If this is the case, the results of the present study support the notion that these EGFR ligands may modulate the PA-plasminogen system in endometrial stroma undergoing decidualization during implantation. Notably, uPA mRNA levels are high in decidualizing stromal cells just after the onset of implantation (Wang et al., 1996).

CONCLUSIONS

In summary, the present study shows that EGF stimulates net uPA activity in the medium of cultured endometrial stromal cells isolated from rat uteri sensitized for decidualization. This effect was prevented or greatly reduced by inhibitors of transcription and translation and was accompanied by an increase in steady-state uPA mRNA levels. Therefore, this study provides evidence that EGF causes an increase in uPA activity in the medium through a mechanism involving an increase in steady-state uPA mRNA levels. Further studies are required to determine if the effect of EGF on net PA activity also can be explained, in part, by decreased secretion of uPA inhibitors such as PAI-1 and PAI-2.

ACKNOWLEDGMENTS

We thank M.A. Shu, H.E. Ross, and G. Barbe for technical assistance. The Medical Research Council of Canada supported this research by Grant MT-10414 to TGK and Grant MT-12107 to XZ, who was also the recipient of an MRC Scholarship Award.

REFERENCES

Bany BM, Kennedy TG (1995): Regulation by epidermal growth factor of prostaglandin production and cyclooxygenase activity in sensitized rat endometrial stromal cells in vitro. J Reprod Fertil 104: 57-62.

Blasi F, Vassalli J-D, Danø K (1987): Urokinase-type plasminogen activator: Proenzyme, receptor, and inhibitors. J Cell Biol 104:801-804.

Chomczynski P, Sacchi N (1987): Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. Anal Biochem 162: 156-159.

Coleman 'PL, Green GDJ (1981): A sensitive, coupled assay for plasminogen activator using a thiol ester substrate for plasmin. Ann NY Acad Sci 370:617-626.

Danø K, Andreasen PA, Grondahl-Hansen J, Kristensen P, Nielsen LS, Skriver L (1985): Plasminogen activators, tissue degradation, and cancer. Adv Cancer Res 44: 139-266.

Das SK, Wang X-N, Paria BC, Damm D, Abraham JA, Klagsbrun M, Andrews GK, Dey SK (1994a): Heparin-binding EGF-like growth factor gene is induced in the mouse uterus temporally by the blastocyst solely at the site of its apposition: A possible ligand for interaction with blastocyst EGF-receptor in implantation. Development 120: 1071-1083.

Das SK, Tsukamura H, Paria BC, Andrews GK, Dey SK (199413): Differential expression of epidermal growth factor receptor (EGF-R) gene and regulation of EGF-R bioactivity by progesterone and estrogen in the adult mouse uterus. Endocrinology 134:971-981.

Das SK, Chakraborty I, Paria BC, Wang X-N, Plowman G, Dey SK (1995): Amphiregulin is an implantation-specific and progesterone-regulated gene in the mouse uterus. Mol Endocrinol 9:691-705.

Finn CA (1986): Implantation, menstruation and inflammation. Biol Rev 61:313-328.

Finn CA, Porter DG (1975): "The Uterus."Acton: Publishing Sciences Group, pp 86-95.

Heussen C, Dowdle EB (1980): Electrophoretic analysis of plasminogen activators in polyacrylamide gels containing sodium dodecyl sulfate and copolymerized substrates. Anal Biochem 102: 196-202.

Jensen PJ, Rodeck U (1993): Autocrine/paracrine regulation of keratinocyte urokinase plasminogen activator through the TGFa/GF receptor. J Cell Physiol 155:333-339.

Johnson DC, Chatterjee S (1993): Epidermal growth factor (EGF) replaces estradiol for the initiation of embryo implantation in the hypophysectomized rat. Placenta 14:429-438.

Lowry OH, Rosebrough NJ, Farr AL, Randall RJ (1951): Protein measurement with the Folin phenol reagent. J Biol Chem 193:265-275.

Miyauchi A, Momoeda M, Nakabayashi M, Osuga Y, Yano T, Tsutsumi 0, Taketani Y (1995): Regulation of the plasminogen activator/plasmin system by epidermal growth factor in cultured human endometrial cells. Hum Reprod 12:3284-3288.

Paria BC, Das SK, Huet-Hudson YM, Dey SK (1994): Distribution of transforming growth factor a precursors in the mouse uterus during the periimplantation period and after steroid hormone treatments. Biol Reprod 50:48 l-49 1.

Psychoyos A (1973): Endocrine control of egg implantation, in RO Greep, EB Astwood, SR Geiger (eds): "Handbook of Physiology," sec 7, vol II, part 2. Washington, DC: American Physiological Society, pp 187-215.

Sambrook K, Fritsch EF, Maniatis T (1989): "Molecular Cloning: A Laboratory Manual." Cold Spring Harbor: Cold Spring Harbor Laboratory Press.

Smith CL, Hammond GL (1991): Ontogeny of corticosteroid-binding globulin biosynthesis in the rat. Endocrinology 128:983-988.

Tamada H, Das SK, Andrews GK, Dey SK (1991): Cell-type-specific expression of transforming growth factor a in the mouse uterus during the peri-implantation period. Biol Reprod 45:365-372.

Tong BJ, Das SK, Threadgill D, Magnuson T, Dey SK (1996): Differential expression of the full-length and truncated forms of the epider-

ma1 growth factor receptor in the preimplantation mouse uterus and blastocyst. Endocrinology 137:1492-1496.

Tranque P, Naftolin F, Robbins R (1994): Differential regulation of astrocyte plasminogen activators by insulin-like growth factor I and epidermal growth factor. Endocrinology 134:2606-2613.

Vassalli J-D, Belin D (1987): Amiloride selectively inhibits the urokinase-type plasminogen activator. FEBS Lett 214:187-191.

Vladimirsky F, Chen L, Amsterdam A, Zor U, Lindner HR (1977): Differentiation of decidual cells in cultures of rat endometrium. J Reprod Fertil 49:61-68.

Wang S, Kennedy TG, Zhang X (1996): Presence of urokinase plasminogen activator and plasminogen activator inhibitor 1 messenger ribonucleic acids in rat endometrium during decidualization in vivo. Biol Reprod 55:493-497.

Weitlauf HM (1994): Biology of implantation, in E Knobil, JD Neil1 (eds): "The Physiology of Reproduction." New York: Raven Press, pp 391-440.

Yee GM, Kennedy TG (1991): Role of cyclic adenosine 3',5'-monophosphate in mediating the effect of prostaglandin E_2 on decidualization in vitro. Biol Reprod 45: 163-17 1.

Zhang X, Shu MA, Ross HE, Kennedy TG (1996): Regulation of plasminogen activator in rat endometrial stromal cells: the role of prostaglandin E_2. Biol Reprod 54:1046-1051.