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Regulation of Cyclooxygenase Gene Expression in Rat Endometrial Stromal Cells: The Role of Epidermal Growth Factor

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ABSTRACT Epidermal growth factor stimulates prostaglandin production and cyclooxygenase activity in endometrial stromal cells isolated from the uteri of ovariectomized rats sensitized for the decidual reaction. The present study examined the effect of epidermal growth factor on cyclooxygenase-1 and cyclooxygenase-2 mRNA and protein levels in these cells. Treatment with epidermal growth factor (40 ng/ml) for 24 hr increased steady-state cyclooxygenase-1 and cyclooxygenase-2 mRNA and protein levels in the cells as determined by Northern and Western analyses. Dexamethasone inhibited the epidermal growth factor induced increases in steady-state mRNA levels for cyclooxygenase-2, but not for cyclooxygenase-1. Finally, the effects of epidermal growth factor and dexamethasone on steady-state cyclooxygenase-1 and cyclooxygenase-2 mRNA levels paralleled the changes in the levels of immunocytochemical staining for these enzymes in the cells. This showed that the changes in cyclooxygenase-1 and -2 protein levels were due to generalized changes in most cells, and not to changes in a subpopulation of stromal cells. The results of this study suggest that epidermal growth factor causes an increase in cyclooxygenase-1 and cyclooxygenase-2 gene expression in endometrial stromal cells isolated from the uteri of rats which have been sensitized for decidualization, and that the previously reported transcriptional- and translational-dependent increases in cyclooxygenase activity in these cells in response to epidermal growth factor were likely due to its effect on cyclooxygenase- 1 and cyclooxygenase-2 gene expression. Dev. Genet. 21:109-115, 1997. © 1997 Wiley-Liss, Inc.

Key words: Epidermal growth factor; cyclooxygenase; uterus; endometrium

INTRODUCTION

Cyclooxygenase (COX) is believed to be a rate-limiting enzyme in the production of prostaglandins [PGs; DeWitt, 1991]. COX converts arachidonic acid sequentially to prostaglandin G_2 and then to prostaglandin H_2 (PGH_2) through bis-oxygenase and peroxidase activities, respectively [Smith *et al.,* 1991]. PGH_2 is then the direct precursor of the biologically active 2-series PGs and thromboxanes. In several cell types, agonist-induced increases in PG production is accompanied by an increase in COX gene expression [DeWitt, 1991; Herschman, 1994]. The level of COX gene expression may be important for prolonged elevated PG production since COX is a suicide enzyme [Egan *et al.,* 1976] and must be replaced to sustain PG production. Recently, it has been realized that two isozymes of COX exist. Two separate human [Hla and Neilson, 1992; Takahashi *et* al., 1992], mouse [DeWitt *et* al., 1991; Kujubu *et* al., 1991], and rat [Feng *et* al., 1993] COX genes have been cloned and are called cyclooxygenase-1 (COX-1) and cyclooxygenase-2 (COX-2). The 4.1 kb COX-2 mRNA is much larger than the 2.8 kb COX-1 mRNA, mainly because of the presence of a large T-untranslated region in COX-2. The most striking difference between these two isoforms is the presence of this large T-untranslated region in COX-2 containing a large number of Shaw-Kamen sequences which are known to confer selective mRNA degradation on other transcripts [Shaw and Kamen, 1986]. Despite the differences in transcript sizes, the COX-1 and COX-2 genes encode proteins of almost identical molecular mass which share approximately 75% sequence identity within species.

PGs, particularly of the E-series, play an important role in blastocyst implantation and decidualization in many animals [reviewed by Kennedy, 1990; Smith, 1991]. PG levels are higher in the endometrium. at implantation sites compared to interimplantation sites in many species. Inhibition of endometrial PG production prevents or delays implantation and decidualization in many species, effects which can be prevented by the administration of exogenous PGs. Although essen

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tial for implantation, the source and control of PG biosynthesis in the endometrium. during implantation are not known. However, epithelial cells may release signals in response to the implanting blastocyst which, in turn, stimulate PG production by endometrial stromal cells. Consistent with this scheme, it is known that EGF receptor (EGFR) ligands are produced mainly by the luminal epithelium [Das *et al*., 1994b, Paria *et al*., 1994; Tamada *et al*., 1991] and are capable of increasing PG production by cultured mouse [Paria *et* al., 1991] and rat [Bany and Kennedy, 1995] endometrial stromal cells. Further, EGFRs are present in mouse endometrial stromal cells during the peri-implantation period [Das *et* al., 1994b; Tong *et* al., 1996]. Therefore, epithelial EGFR ligands may play a role in controlling PG production in endometrial stromal cells during implantation.

A previous study showed that EGF stimulates PG production by endometrial stromal cells isolated from rat uteri sensitized for the decidual cell reaction [Bany and Kennedy, 1995]. This increase in PG production was accompanied by a transcription- and translation dependent increase in COX activity. Since COX is a rate-limiting enzyme in PG production [DeWitt, 1991], the purpose of the present study was to extend these findings to determine the effect of EGF on the expression of COX-1 and COX-2 genes in these cultured rat endometrial stromal cells.

MATERIALS AND METHODS

Animals

Female Harlan Sprague-Dawley rats (200-225 g), obtained from Harlan Sprague-Dawley Inc. (Indianapolis, IN), were housed under temperature- and light controlled conditions (14 hr lights on: 10 hr lights off; 1200 hr as the midpoint of the light phase) with free access to food and water. The animals were ovariectomized under ether anesthesia and allowed at least 6 days to recover. To obtain rats with uteri sensitized for decidualization, estradiol and progesterone in sesame oil were administered subcutaneously (s.c.) as described elsewhere [Bany and Kennedy, 1995].

Endometrial Stromal Cell Isolation and Culture

Rats were killed by decapitation and the endometrial stromal cells were isolated by a modification of the methods of McCormick and Glasser [1980] as described in detail elsewhere [Bany and Kennedy, 1995]. Briefly, uterine horns were obtained, dissected free from adherent tissue, then split longitudinally. The uterine horns were initially incubated with pancreatin (GIBCO-BRL, Burlington, ON, Canada) and dispase II (Boehringer Mannheim, Laval, PQ Canada) to liberate luminal epithelial cells, then in type II collagenase (Sigma Chemical Co., St. Louis, MO) to liberate endometrial stromal cells. The stromal cells were suspended in Dulbecco's modified Eagle's medium:F12 Nutrient Mixture (DMEM:F12) containing 10% heat-inactivated charcoal-stripped fetal calf serum, 50 units/mI penicillin, 50 μg/ml streptomycin, and 1.25 μg/mI fungizone (all GIBCO-BRL). The cell suspension was filtered through nylon mesh (70 μm) to remove glands and clumps of epithelial cells and plated at $5 X 10^5$ cells (in 0.5 ml culture medium) in 24-well plates (BectonDickinson, Lincoln Park, NJ) with or without Thermanoxg coverslips (Nunc Inc., Naperville, IL). After an initial incubation period of 2 hr at 37°C under 5% C02:95% air to allow differential attachment of the stromal cells, the medium and free floating cells were removed and replaced with serum-free DMEM:F12. This was defined as 0 hr of day 1 of culture. The resultant cultures were essentially free of epithelial cells as indicated by the absence of positively staining cells for cytokeratin by immunocytochemistry (data not shown).

Cells were treated with or without human recombinant EGF (GIBCO-BRL). Previous results have shown that EGF has it greatest stimulative effect on PGE_2 accumulation when added to the culture medium for 24 hr on day 3 of culture [Bany and Kennedy, 1995]. Therefore, the experiments were carried out on day 3. Dexamethasone (Sigma), prepared as a stock at a concentration of 10 mM in ethanol, was used in some experiments. Since a final concentration of 5 μM of dexamethasone was used, all treatments contained 0.1% ethanol in experiments involving dexamethasone

Northern Blot Preparation and Hybridization

Total RNA was isolated from the cells using a single step guanidine thiocyanate procedure [Chomczynski and Sacchi, 1987]. Total RNA was quantified by absorbance at 260 nm as well as by ethidium bromide staining after electrophoresis through agarose gels. Samples of total RNA (10 pg per lane) were denatured in 6% formaldehyde:50% formamide in MOPS buffer (10 mM MOPS, 5 mM sodium acetate, 1 mM EDTA, pH 7.2) at 65°C for 15 min, subjected to denaturing agarose gel (1.1%) electrophoresis, and then transferred to Hybond-N membranes (Amersham, Oakville, ON) by capillary transfer as previously described [Sambrook et al., 1989]. The RNA was crosslinked to the membranes by UV irradiation ($0.12 J/cm^2$).

Probes for COX-1 and COX-2 were prepared from EcoRI digests of full-length rat cDNAs kindly provided by Dr. L. Feng (Scripps Research Institute, La Jolla, CA). The 1,197 bp COX-1 and 812 bp COX-2 EcoRI cDNA fragments (25 ng) were labeled using a random primer DNA labeling system (GIBCO-BRL) in the presence of ^{32}P-dCTP (Amersham) and purified using Nick Columns (Pharmacia Biotech, Baie d'Urfé, PQ) according to the manufacturer's instructions. Membranes were incubated in prehybridization buffer (7% sodium dodecyl sulfate [SDS], 0.5 M Na_2HPO_4, 0.1 mM EDTA, 1% bovine serum albumin, pH 7.2) for 30 min at 65°C. Labeled probe (specific activity = 1 mCi/mg), denatured in 0.4 M NaOH for 5 min, was added and

hybridization was carried out overnight at 60°C. The membranes were then washed three times for 15 min in wash buffer (1% SDS, 40 mM NaH_2PO_4, pH 7.2) at 65°C and subjected to autoradiography at -70°C with XOmat AR film (Kodak) and intensifying screens. Probes were removed between hybridizations in stripping buffer (1 mM Tris, 1 mM EDTA, 0.1 strength Denhardts' reagent [Sambrook *et al.*, 1987], pH 8.0) for 2 hr at 75°C.

The relative amounts of RNA loaded into each lane and transferred to the membranes were determined by probing the blots with ^{32}P-labeled cDNA for mouse 18 S rRNA [Smith and Hammond, 1991]. COX-1 and COX-2 mRNA along with 18S rRNA signals were quantified by densitometry. The relative levels of the signals for the mRNAs on the autoradiograms were quantified by densitometry and the results were expressed as the ratios of mRNA signal to 18S rRNA signal.

Western Blots

Cells were washed with phosphate-buffered saline (PBS), scraped into boiling lysis buffer (1% SDS, 10 mM Tris, pH 7.4), and boiled for 5 min. After centrifugation at 12,000g for 5 min to remove insoluble material, protein concentration in the sample was determined using the Bio-Rad DC Protein Assay (Bio-Rad Laboratories, Mississauga, ON). Protein samples (50 μg) and prestained Rainbow Molecular Mass Markers (Amersham) were subjected to 10% SDS-polyacrylamide gel electrophoresis (PAGE) under reducing conditions [Laemmli, 1970], and then transferred to Immobilon™-P membranes (Millipore Corporation, Bedford, MA) by electroblotting [Towbin *et al.*, 1979]. The membranes were placed in blocking buffer (5% nonfat dry milk in TBS: 10 mM Tris, pH 7.5, 100 mM NaCl, 0.1% Tween-20) for 1 hr and then in blocking buffer containing affinity-purified COX-1 or COX-2 goat antibodies (0.2 μg/ml; Santa Cruz Biotechnology, Santa Cruz, CA) for 1 hr. After six 5-min washes in TBS, the membranes were incubated with peroxidase-conjugated donkey antigoat IgG (80 ng/ml; Jackson ImmunoResearch Laboratories, West Grove, PA) for 1 hr. After washing in TBS, COX-1 or COX-2 was detected by enhanced chemiluminescence using an Ahermsham-ECL Kit (Amersham). Densitometry was carried out to determine changes in COX-1 and COX-2 levels.

Immunocytochemistry

Cells grown on coverslips were rinsed in PBS (0.01 M phosphate, 0.9% NaCl, pH 7.4), fixed in ice-cold methanol for 5 min, air dried, then rehydrated in PBS. To quench endogenous peroxidase activity, the cells were incubated in 0.1% hydrogen peroxide in PBS for 5 min. Cells were then blocked with PBS containing 5% normal donkey serum (PBS-NDS) for 30 min at room temperature followed by an overnight incubation at 4°C with affinity-purified goat antibodies against rat COX-1 or COX-2 (0.1 μg/ml; Santa Cruz Biotechnology) in PBS-NDS. After three 10-min washes in PBS, the cells were incubated with biotinylated donkey anti-goat IgG (1.8 μg/ml) in PBS-NDS for 1 hr, washed in PBS, then incubated with peroxidase-conjugated streptavidin (1 μg/ml; Jackson ImmunoResearch Laboratories) in PBS for 30 min. After three 5-min washes in water, immunoreactive COX-1 or COX-2 in the cells was detected as a red color after incubation with 3-amino-9-ethyl-carbazole (AEC Substrate Kit, Dimension Laboratories, Inc., Mississauga, ON, Canada). Special care was taken to ensure that all coverslips were incubated with the substrate for exactly 5 min followed by washing with PBS to stop the reaction. To determine the specificity of the immunolocalization for each antibody, some coverslips were incubated with primary antibody preincubated for 2 hr at room temperature with a 10-fold excess by weight of control peptide (the peptide used to generate the antibodies).

Statistical Analysis

Analysis of variance (ANOVA) was used to determine treatment effects and where significant ($P < .05$) interactions were detected, Duncan's multiple range test was used for group comparisons. All statistical analysis was carried out using SigmaStat Software (Jandel Scientific, Corte Madera, CA).

RESULTS

Effect of EGF on COX-1 and COX-2 Gene Expression

Northern blot analyses were used to determine the effects of EGF on the steady-state levels of mRNAs for COX-1 and COX-2 in the cultured cells (Fig. 1). Cells were incubated with or without EGF (40 ng/ml) for 24 hr on day 3 of culture and the steady-state levels of COX-1 and COX-2 mRNAs were determined. A single species of mRNA of approximately 2.8 and 4.1 kb was detected when the blots hybridized with the ^{32}P-labeled COX-1 and COX-2 probes, respectively (Fig. 1a). COX-1 and COX-2 mRNA levels were barely detectable in vehicle-treated cells. However, after incubation with EGF, the abundance of COX-1 and COX-2 mRNAs relative to 18S rRNA levels increased significantly ($P < .05$) by 4-fold and 3.8-fold, respectively (Fig. 1b).

Western blot analyses were used to determine the effect of EGF on the levels of immunoreactive COX-1 and COX-2 proteins in the cultured cells. Cells were incubated with or without EGF (40 ng/ml) for 24 hr on day 3 of culture and the levels of immunoreactive COX-1 and COX-2 proteins were determined. For COX-1 and COX-2, proteins of approximate molecular mass of 68 kDa were detected (Fig. 2a). COX-1 and COX-2 protein were detected in the cells incubated with vehicle alone for 24 hr on day 3 of culture. EGF (40 ng/ml) significantly increased the levels of immunoreactive COX-1 ($P < .02$) and COX-2 ($P < .002$) protein (Fig. 2b). Blots incubated with COX-1 and COX-2 primary antibodies preincubated with control peptides showed no bands (data not shown).

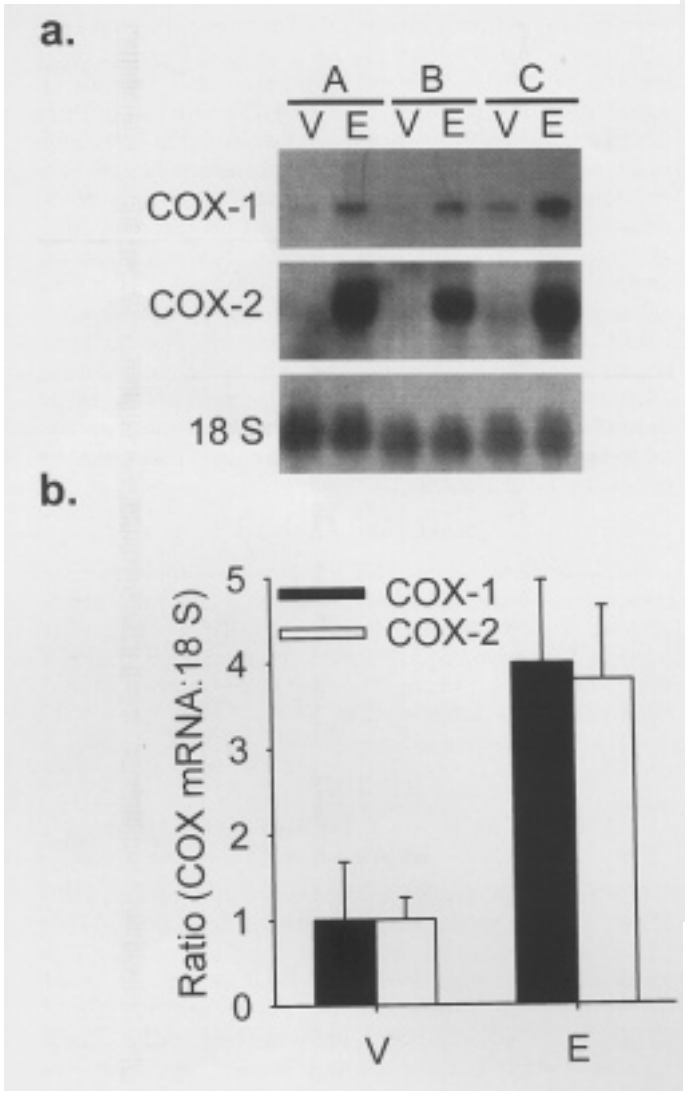

Fig. 1. Northern analysis of COX-1 and COX-2 mRNA from rat endometrial stromal cells cultured with vehicle (V) or EGF (E; 40 ng/ml) for 24 hr on day 3 of culture. **a:** Autoradiograph of a membrane containing samples from three separate experiments (A-C). The membrane was hybridized with ^{32}P-labeled COX-1, COX-2, and 18S rRNA cDNA probes. **b:** Mean (±SEM, N = 3) ratio of mRNA/18 S rRNA signals, as determined by image analysis with the ratios for vehicle-treated cells set to 1 for both groups.

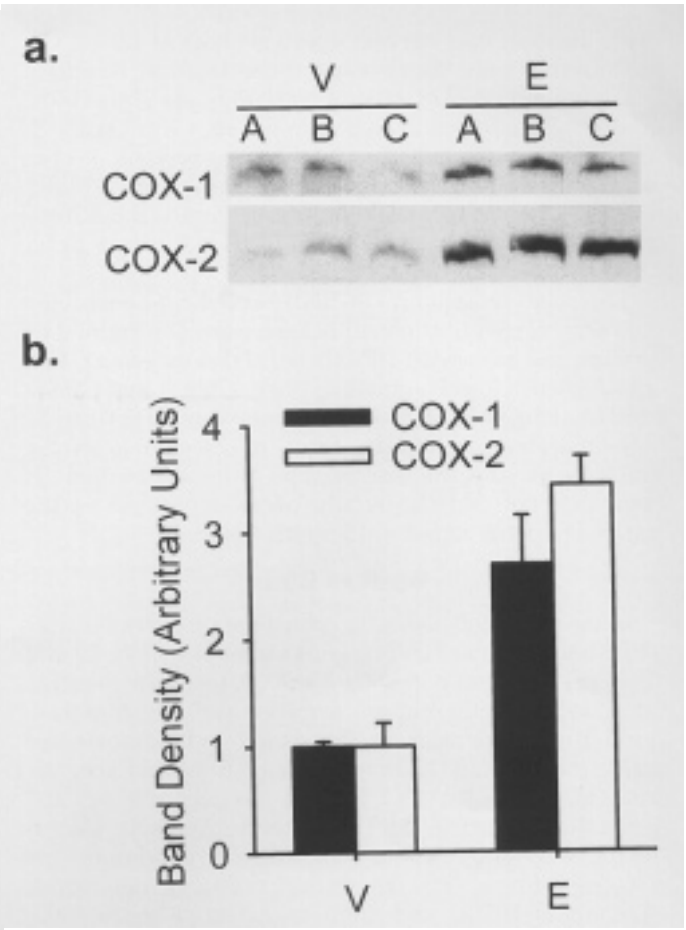

Fig. 2. Western analysis of COX-1 and COX-2 proteins in rat endometrial stromal cells cultured with vehicle (V) or EGF (E; 40 ng/ml) for 24 hr on day 3 of culture. **a:** Results from two membrane containing samples (50 μg total protein per lane) from three separate experiments (A-C). One membrane was incubated with COX-1 antibody and the other was incubated with COX-2 antibody. **b:** Mean (± SEM, N = 3) density of immunoreactive protein signals, as determined by image analysis, with the ratios for vehicle-treated cells set to 1 for both groups.

Effect of Dexamethasone on the EGF-Induced Increases in COX-1 and COX-2 Gene Expression

Cells were incubated with or without EGF (40 ng/ml) in the presence or absence of dexamethasone (5μM) for 24 hr on day 3 of culture and the steady-state levels of COX-1 and COX-2 mRNAs (Fig. 3) were determined. For steady-state COX-1 mRNA levels, ANOVA revealed significant ($P < .05$) effects of EGF and dexamethasone, without a significant ($P > .05$) interaction. Thus, EGF stimulated and dexamethasone decreased steady-state levels of COX-1 mRNA. By contrast, ANOVA of the COX-2 data revealed a significant ($P < .05$) interaction between the effects of EGF and dexamethasone. By itself, EGF increased COX-2 steady-state mRNA levels, but this increase was prevented when dexamethasone was present. By itself, dexamethasone decreased COX-2 steady-state mRNA levels. The effect of dexamethasone on the levels of immunoreactive COX-1 and COX-2 proteins was also investigated. However, COX-1 and COX-2 proteins were not detectable in cells treated with dexamethasone or dexamethasone plus EGF (data not shown).

COX-1 and COX-2 Immunocytochemistry

Immunocytochemistry was performed on the cells to determine possible qualitative effects of EGF and dexamethasone on COX-1 and COX-2 protein immunostaining. For all treatments, COX-1 and COX-2 staining was found in all cells (Fig. 4). After incubation with EGF (40 ng/ml), the level of staining in all cells appeared to

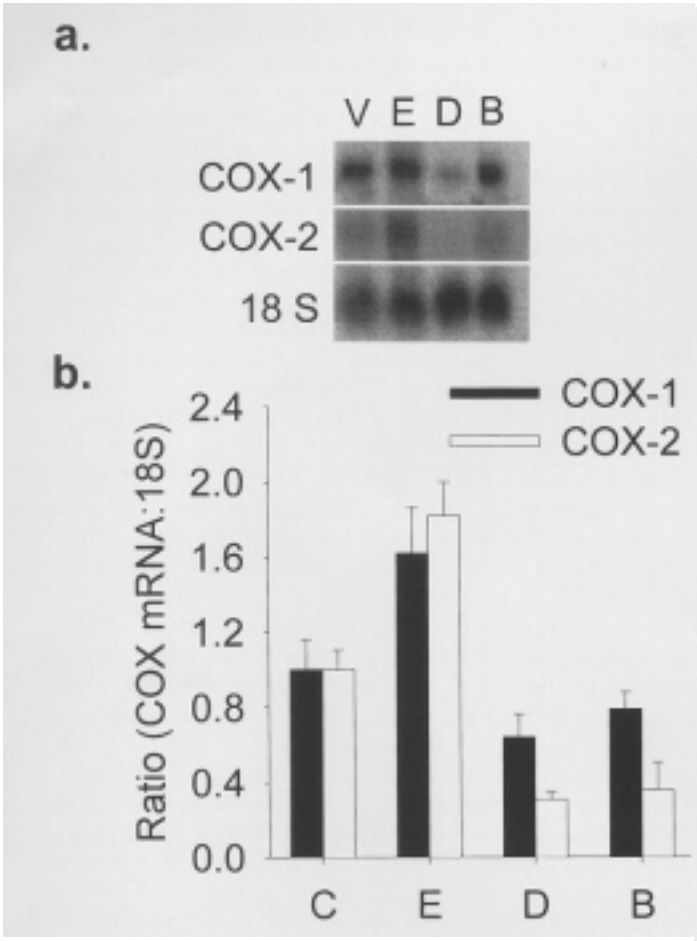

Fig. 3. Northern analysis of COX-1 and COX-2 mRNA from rat endometrial stromal cells cultured with vehicle (V) or EGF (E; 40 ng/ml), dexamethasone (D; 5 μM), or both EW plus dexamethasone (B) for 24 hr on day 3 of culture. **a:** Representative autoradiographs of a membrane containing samples from a single culture. The membrane was hybridized with ^{32}P-labeled COX-1, COX-2, and 18S rRNA cDNA probes. **b:** Mean (± SEM, N = 5) ratio of mRNA/18S rRNA signals, as determined by image analysis with the ratios for vehicle-treated cells set to 1 for all four groups.

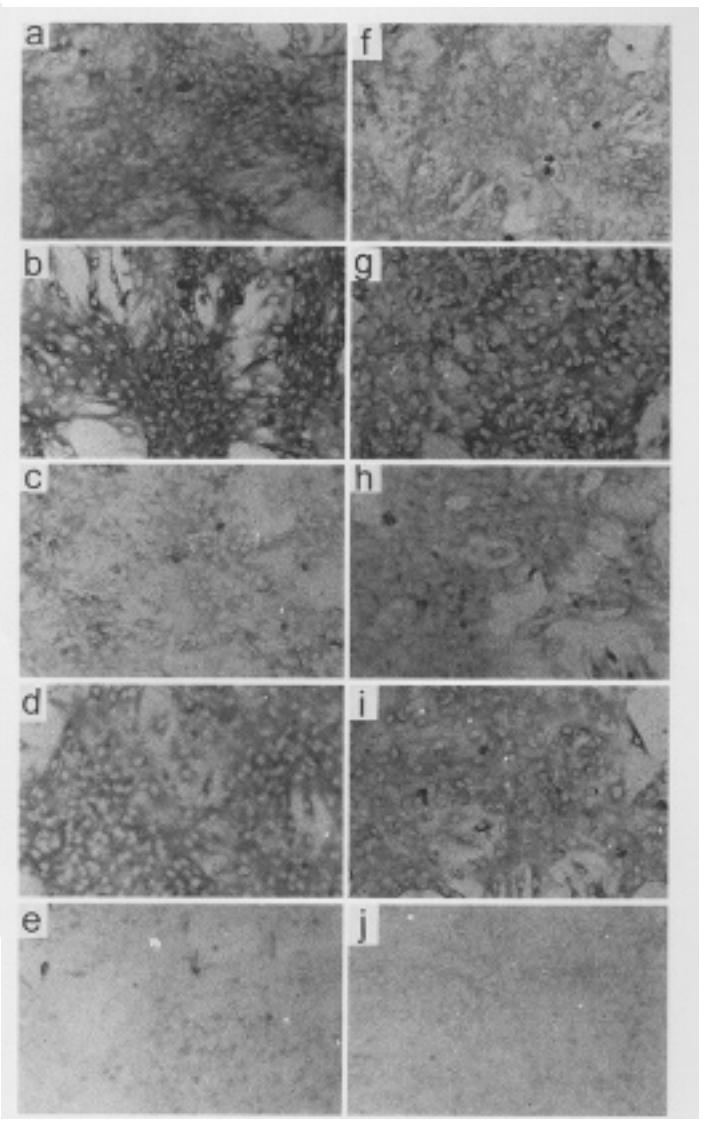

Fig. 4. Photomicrographs of rat endometrial stromal cells immunocytochemically stained with antibodies against COX-1 (a-e) and COX-2 (f-j) proteins. Cells were incubated with vehicle (**a,f**); EGF, 40 ng/ml (**b,g**); dexamethasone, 5 μM (**c,h**); or both EGF plus dexamethasone (**d,i**) for 24 hr on day 3 of culture. Preincubation with control peptide (**e,j**) resulted in the loss of most staining.

increase for both COX-1 and COX-2 (Fig. 4b,g) compared to vehicle-treated cells (Fig. 4a,f). Dexamethasone appeared to decrease COX-1 but not COX-2 staining in all cells compared to vehicle-treated cells. However, in the presence of dexamethasone, EGF caused an increase in staining for COX-1, but not for COX-2 staining in most cells. Preadsorption of the primary antibody with control peptide, at a 10-fold excess by weight, caused loss of most staining (Fig. 4e,j).

DISCUSSION

The present study shows that EGF increases steady-state mRNA and protein levels of COX-1 and COX-2 in rat endometrial stromal cells isolated from uteri sensitized for the decidual cell reaction. The effects of EGF on COX-1 and COX-2 protein levels paralleled changes in the immunocytochemical staining in these cells, showing that the changes in COX-1 and COX-2 protein levels occurred in most cells and not a small subpopulation of stromal cells. We have previously shown that EGF causes transcription- and translation-dependent increases in PG production and COX activity in endometrial stromal cells isolated from rat uteri sensitized for decidualization [Bany and Kennedy, 1995]. The results of the present study suggest that the EGF-induced increase in PG production and COX activity in these cells is due, at least in part, to an increase in COX gene expression. EGF may also increase mRNA and protein stability of COX-1 and COX-2, but further studies are required to determine if such effects of EGF exist.

Although both COX-1 and COX-2 gene expression can be induced by some agonists, COX-1 gene expression is not induced in as many cell types and is usually induced to a lesser extent compared to COX-2 [Herschman, 1994]. In the present study, both COX-1 and COX-2 mRNA and protein levels were increased by EGF, suggesting that both genes are induced in rat endometrial stromal cells isolated from uteri sensitized for the decidual cell reaction. However, both COX-1 and COX-2 were expressed in the endometrial stromal cells under control conditions, as shown by immunocytochemical, Western, and Northern analyses. Whether this expression of both COX-1 and COX-2 under control conditions is a response to endogenously produced agonists (possibly EGFR ligands acting in an autocrine manner), or alternatively reflects constitutive expression, remains to be determined.

Dexamethasone decreases PG production and COX activity in rat endometrial stromal cells in the presence or absence of EGF [Bany and Kennedy, 1995]. In the present study, dexamethasone decreased both COX-1 and COX-2 steady-state mRNA and protein levels in the presence or absence of EGF. This suggests that the effects of dexamethasone on PG production and COX activity were due, at least in part, to an inhibition of both COX-1 and COX-2 gene expression. The extent to which these in vitro observations may explain the effects of dexamethasone on blastocyst implantation is unclear. In rats, dexamethasone inhibits estradiol induced implantation and this can be reversed by PG treatment [Johnson and Dey, 1980]. Further, dexamethasone inhibits estradiol- and EGF-induced implantation in hypophysectomized rats undergoing delay of implantation [Johnson and Chatterjee, 1995]. These observations are consistent with the notion that dexamethasone inhibited COX expression, thereby reducing the PG production which is required for implantation. However, in the mouse, dexamethasone affects neither implantation nor the normal expression of COX-2 in the uterus during estradiol-induced implantation [Chakraborty *et al.,* 1996]. Therefore, the physiological role of glucocorticoids in regulating endometrial COX gene expression and PG production in vivo is not clear and may be species specific.

EGFR ligands may play an important role in implantation. EGFR ligands increase in a cell- and time--dependent manner in the mouse uterus and blastocyst during the peri-implantation period [Tamada *et al.,* 1991; Das *et al.,* 1994a; Paria *et al.,* 1994] and increase during in vitro decidualization of human endometrial stromal cells [Sakakibara *et al.,* 1994; Taga *et al.,* 1995]. Further, the number of EGFRs increase in the uterus during implantation [Chakraborty *et al.,* 1988; Brown *et al.,* 1989] and are localized to all cell types, except the luminal and glandular epithelia [Das *et al.,* 1994b] which express a truncated form [Tong *et al.,* 1996]. Finally, EGF induces implantation of embryos transferred into hypophysectomized rats [Johnson and Chatterjee, 1993] and enhances decidualization [Tamada *et al.,* 1994]. Although these studies provide indirect and direct evidence that EGFR ligands may play a role in the endometrium during the peri-implantation period, the precise role of EGFR ligands remains speculative. The results of the present study raise the possibility that EGFR ligands may function to regulate COX-1 and COX-2 gene expression and thus PG production in the endometrial stroma during the peri-implantation period.

COX-1 and COX-2 are expressed in a time- and cell-specific manner in the mouse endometrium during implantation. The expression of COX-1 appears mainly in the uterine epithelial cells in mice [Jacobs *et al.,* 1994; Chakraborty *et al.,* 1996]. Since the epithelial cells appear not to express functional EGFRs Was *et al.,* 1994b; Tong *et al.,* 1996], it is unlikely that EGFR ligands regulate COX-1 gene expression in these cells. On the other hand, COX-2 mRNA and protein appear in the subepithelial stroma and transiently in the luminal epithelium in the immediate vicinity of the implanting embryo during the early phase of implantation in the mouse [Chakraborty *et al.,* 1996; Jacobs *et al.,* 1995]. This expression of COX-2 protein in the subepithelial stroma continues in the primary and secondary decidual zones [Jacobs *et al.,* 1996]. The expression of COX-2 correlates with the expression of EGFR ligands in the luminal epithelium [heparin-binding (HB)EGF, Das *et al.,* 1994a; EGF, Huet-Hudson *et al.,* 1990; transforming growth factor alpha (TGFα), Paria *et al.,* 1994; Tamada *et al.,* 1991] and decidualizing stroma [TGFα, Bonvissuto *et al.,* 1992]. From the results of the present study, it can be speculated that these EGFR ligands may serve to induce COX-2 gene expression and thereby contribute to the increase in PG levels found at implantation sites. However, the role of EGFR ligands in the control of COX-2 gene expression and PG production in the endometrium in vivo during implantation remains speculative and more work is required. COX-1 and COX-2-deficient mice may provide a tool for studying the importance of COX-1 and COX-2 in the implantation and control of endometrial PG production. Notably, COX-1 gene expression is not absolutely required for implantation in the mouse since COX-1 deficient mice are fecund [Langenbach *et al.,* 1995]. On the other hand, COX-2-deficient mice are infertile, but this appears to be primarily due to a defect in ovulation and the lack of copora lutea in the ovary [Dinchuk *et al.,* 1995]. Finally, COX-1- and COX-2-specific inhibitors are potential tools in addressing the roles of COX-1 and COX-2 in the uterus during implantation.

In summary, EGF causes an increase in steady-state COX-1 and COX-2 mRNA and protein levels in endometrial stromal cells isolated from uteri of ovariectomized rats which had been sensitized for decidualization. The EGF-induced increase in COX-2 mRNA and protein levels are prevented by dexamethasone. Although dexamethasone decreases basal levels of COX-1 mRNA, it does not effect the EGF-induced increase in COX-1 mRNA. From these results, we conclude that increases in COX-1 and COX-2 mRNA and protein levels in

response to EGF explain, at least in part, previous reports that EGF stimulates COX activity in rat endometrial cells.

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