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Role of EP2 Receptors and cAMP in Prostaglandin E_2 Regulated Expression of Type I Collagen α1, Lysyl Oxidase, and Cyclooxygenase-1 Genes in Human Embryo Lung Fibroblasts

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Abstract In a recent communication, we demonstrated that prostaglandin E_2 (PGE_2) lowers basal while it ablates interleukin-1β((IL-1β) and transforming growth factor-β (TGFβ) upregulated lysyl oxidase (LO) mRNA levels. Correspondingly, PGE_2 increases cyclooxygenase-1 (COX1) mRNA in diploid, human embryo lung fibroblasts (IMR90) [Roy et al., 1996]. We now report that these actions by PGE_2 are routed through cAMP via the PGE_2, EP2 receptor. Among the PGE_2 receptor types, the IMR90 predominantly express the EP2 mRNA. These cells also express EP3 and EP4 mRNA at comparatively low levels. Northern blot analyses show that 11-deoxy PGE_1, an EP2/EP4 agonist, emulates the action of PGE_2. In a similar manner to PGE_2, 11-deoxy PGE_1 decreases basal and TGF-β induced type I collagen α1 (COL) mRNA, basal and IL-1β induced LO mRNA while it increases COX1 mRNA. Sulprostone, an EP3/EP1 agonist, has no effect on the expression of these three genes. Forskolin, an adenylate cyclase activator, acts in a very similar manner to PGE_2 or 11-deoxy PGE_1. It suppresses both basal and TGF-β induced COL mRNA levels. Both PGE_2 and 11-deoxy PGE_1 increase cAMP to a level comparable with forskolin. The role of the EP2 receptor in controlling collagen production is further underscored in the immortalized Rat-1 fibroblasts, derived from Fischer rat embryos, which do not express detectable EP2 mRNA. In these cells, PGE_2 has little effect on COL mRNA level, whereas forskolin increases it. Furthermore, forskolin increases cAMP level in Rat-1 cells, whereas PGE_2 does not. Overall, these results illustrate that much of the PGE_2 action on the expression of COL, LO, and COX1 genes is mediated through the EP2 receptor and a subsequent increase in intracellular cAMP. J. Cell. Biochem. 71:254–263, 1998.

Key words: lysyl oxidase; cyclooxygenase-1; type I collagen α1; prostaglandin E_2; prostaglandin E_2 receptors; cyclic AMP; interleukin-1β; transforming growth factor-β; forskolin; 11-deoxy PGE_1

An important aspect in the pathology of inflammation, injury, and fibrotic disease is the excess production and fibrous deposition of collagen. Limiting the fibrotic response is crucial in limiting scar formation and tissue fibrosis. Prostaglandin E_2 (PGE_2) exerts such antifibrotic activity. This prostanoid has been shown to inhibit type I collagen α1 (COL) production in IMR90 under both basal and transforming growth factor-β (TGF-β) stimulated cell conditions [Fine et al., 1989]. Prostaglandin E_2 also inhibits collagen synthesis in human cardiac fibroblasts [Brilla et al., 1995], growth plate chondrocytes [O'Keefe et al., 1992], and osteoblasts [Raisz et al., 1993].

The deposition of collagen into extracellular connective tissue matrix requires the presence of lysyl oxidase (LO). We showed previously that PGE_2 inhibits the expression of LO, whereas it promotes the expression of cyclooxygenase-1 (COX1) in IMR90 cells [Roy et al., 1996]. PGE_2 also abrogates interleukin-1β (IL-1β) and TGF-β stimulated LO gene expression [Boak et al., 1994; Roy et al., 1996]. Thus, PGE_2 potentially forms the core of a cycle (PGE_2-collagen), which regulates the production and deposition of collagen at times of injury and inflammation.

The diversified biological effects of PGE_2 are primarily exerted in an autocrine fashion via four receptor types, EP1, EP2, EP3, and EP4 [Coleman et al., 1994; Ichikawa et al., 1996]. In this report, we determine the distribution of

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the EP receptor types expressed in IMR90. We identify the receptor type that interacts with PGE_2 to alter the expression of COL, LO, and COX1 in IMR90. We illustrate the role of cAMP in this process.

MATERIALS AND METHODS

Materials

PGE_2 and forskolin were purchased from Sigma (St Louis, MO); IL-1β was purchased from Genzyme (Cambridge, MA); sulprostone and 11-deoxy PGE_1 were purchased from Cayman Chemical Co (Ann Arbor, MI); and TGF-β was purchased from R & D Systems (Minneapolis, MN). EP1 cDNA was a gift from Dr. C.D. Funk (University of Pennsylvania); EP2 and EP3 cDNAs were gifts from Dr. J. W. Regan (University of Arizona); EP4 cDNA and COL cDNA were gifts from Dr. E. J. Goetzl (University of California Medical Center, San Francisco) and Dr. R.H. Goldstein of Boston University School of Medicine, respectively. Human COX1 cDNA was a gift from Dr. T. Hla (Holland Laboratory, American Red Cross).

Cell Culture

IMR90 cells were obtained from Coriell Institute for Medical Research (Camden, NJ). The cells were grown to confluence on 100x20 mm Falcon tissue culture plates for Northern blot experiments and in 12-well plates for cAMP assay. These cells were cultured at 37°C in Minimum Essential Medium Eagle (MEM; Mediatech, Herndon, VA) containing 10% fetal bovine serum (FBS; Sigma) supplemented with 50 units/ml penicillin and 50 µg/ml streptomycin. Cells were used at population doubling level 18 to 30. Before initiation of experiments, the cells were maintained in MEM containing 0.5% FBS for 24 h and incubated with or without test agents for 18 h.

Rat-1 cells were obtained from Dr. Robert Weinberg (Whitehead Institute, MIT) as a gift. Cells were grown to confluence in 100x20 mm sterile Falcon tissue culture plates at 37°C in Dulbecco's Modified Eagle Medium (DMEM; Life Technologies Grand Island, NY) containing 5% FBS supplemented with 50 units/ml penicillin and 50 µg/ml streptomycin. The cells were incubated with or without (control) test agents for 18 h. Prostaglandin E_2, 11-deoxy PGE_1, and sulprostone were dissolved in 100% ethanol, forskolin in dimethyl sulfoxide (DMSO; Sigma), TGF-β (1–2 µg/ml final solution) in 1 mg/ml bovine serum albumin (BSA) containing 4 mM HCl, and IL-1β in phosphate-buffered saline, 0.1% BSA.

Northern Blot Analyses

Total RNA was extracted from cells by guanidinium thiocyanate -phenol-chloroform procedure as described previously [Taylor et al., 1992]. RNA was quantitated by ultraviolet absorbance at 260 nm; 10 µg of total RNA was fractionated by electrophoresis on a 1% agarose and 8% formaldehyde denaturing gel. The fractionated RNA was transferred overnight to nylon membranes from NEN Life Science Products (Boston, MA) and fixed by ultraviolet crosslinking. Approximately 30 ng of cDNA probes for EP receptors (EP1, EP2, EP3, and EP4), COL, LO, and COX1 were labeled with ^{32}P using Ready-to-Go DNA Labeling beads (dCTP) from Pharmacia Biotech (Piscataway, NJ). The RNA blots were probed with given ^{32}P-labeled cDNA at 65°C in a Rapid-hybridization buffer from Amersham Life Science (Amersham, UK) and washed with 2x SSC buffer at 28°C for low stringency, then with 0.2x SSC buffer at 50°C for high stringency. Radioactivity associated with each band was visualized by autoradiography with an intensifying screen at -80°C and quantitated in units of cpm with an Instantimager, (Packard, Meriden, CT). All incubations for Northern blots were done in duplicate. The cpm values were corrected to eliminate loading errors as follows. The ethidium bromide stained 18s rRNAs were scanned through a video snapshot program (Snappy, Play, Rancho Cordova, CA). The intensities of the ethidium bromide stained 18s rRNAs were measured using a Sigma Scan/Image Program (Jandel Scientific, San Rafael, CA). The cpm values were then divided by the 18s rRNA values. To normalize the results, values were expressed as either % of control or fold increase.

Cyclic AMP Assay

Cyclic AMP was quantified as described by Johnson et al. [1994]. The cells were labeled with ^3H-adenine for 24 h, washed with DMEM, buffered with 20 mM HEPES at pH 7.5, and incubated for 30 min in 20 mM HEPES buffered DMEM containing 100 mM 3-isobutyl-1-methylxanthine (IBMX), with or without test agents. Reactions were stopped by aspirating the medium and adding 1 ml ice cold 5% TCA contain-

ing 1 mM ATP and 1 mM cAMP. After a 30 min incubation on ice, the TCA extract was collected and applied to Dowex columns containing analytical grade resin, AG 1-x8 resin (100–200 mesh formate form) from Bio-Rad Laboratories. The columns were washed with 3 ml of water. A total volume of 4 ml was collected in scintillation vials, which represented the ^{3}H-ATP and ^{3}H-ADP fractions. Cyclic AMP was then eluted from the Dowex column directly onto Alumina column (Sigma) with 10 ml water. Cyclic AMP was eluted from the Alumina with 6 ml 0.1 M imidazole, pH 7.5. The radioactivity of the cAMP fraction was quantified with liquid scintillation spectrometry.

RESULTS

Effect PGE_2 Concentration on COL mRNA Levels

A PGE_2 concentration-dependent reduction of COL mRNA steady-state levels in IMR90 cells was first determined. As illustrated in Figure 1, an evident reduction of COL mRNA occurred at 10^{-9}M PGE_2. A stepwise decrease in COL mRNA level was then observed at 10^{-8} and 10^{-7}M PGE_2. Maximal inhibitory effect by PGE_2 took place at 10^{-6}M.

PGE_2 Receptors Expressed in IMR90 Cells

Four PGE_2 receptor subtypes have been identified. To begin to determine the role of these EP

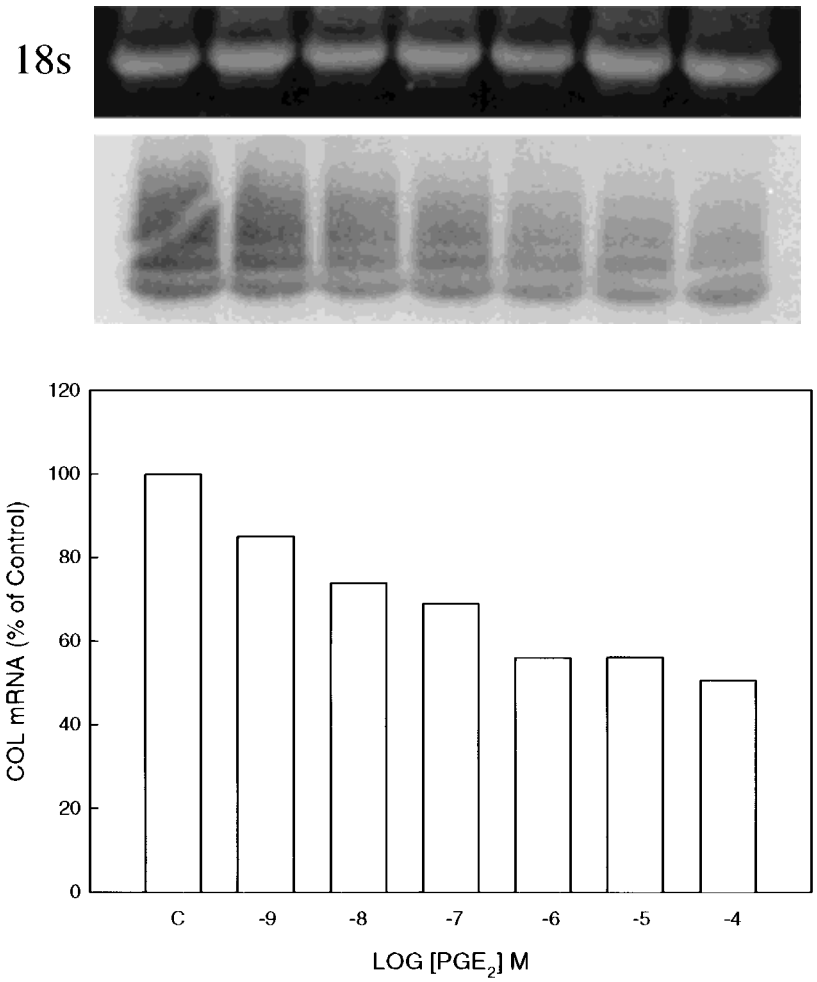

Fig. 1. Effect of increasing concentrations of PGE_2 on COL mRNA. IMR90 cells were made quiescent as described in Materials and Methods. The cells were then treated with the respective concentrations of PGE_2 for 18 h. Total RNA isolated from two separately treated 100 × 20 mm culture plates under the same conditions were combined; 10 µg of total RNA was fractionated by electrophoresis and transferred to nylon membrane as described in Materials and Methods. Then, RNA was hybridized with COL cDNA. C = control (untreated culture), PGE_2 concentration is expressed as log of molar concentration.

receptors in PGE_2 regulation of COL, LO, and COX1 gene expression, receptor mRNA steady-state levels were determined in IMR90 cells. Expression of EP1, EP2, EP3, and EP4 receptors was determined by Northern blot analyses. These results are shown in Figure 2.

The EP2 receptor is clearly predominant in these cells. The EP4 and EP3 receptor mRNAs are detected at a much lower level. The EP1 receptor was not detected. Narumiya [1994] reported similar results in mouse lung with the expression of EP2 mRNA as predominant in comparison to the other PGE_2 receptor subtypes.

Effect of EP2 Agonist, 11-deoxy PGE_1

Agonists for EP2/EP4 and EP3/EP1 were then used to identify the receptor(s) that regulate the expression of the COL, LO, and COX1 genes. As illustrated in Figure 3a, 11-deoxy PGE_1 (1 μM), an EP2/EP4 agonist, affected the expression of all three genes. The agonist decreased steady-state levels of both COL and LO mRNA species and increased COX1 mRNA. In contrast, sulprostone, an EP3/EP1 agonist, had no effect on the steady- state mRNA level of any of the three genes, as illustrated in Figure 3b.

Effect of Forskolin in Comparison to PGE_2

Both EP2 [Regan et al., 1994; Scutt et al., 1995] and EP4 receptors [An et al., 1993; Bastien et al., 1994] have been linked with the cAMP signaling path. Forskolin, an adenylate cyclase activator, was used to determine the effect of increased cAMP levels on COL gene expression. Figure 4 illustrates that forskolin (25 μM), reduced COL mRNA by ~50%. A similar reduction in COL mRNA is observed in response to PGE_2 (1 μM). Interestingly, 1 μM PGE_2 and 25 μM forskolin in combination show a greater inhibition than either PGE_2 or forskolin alone (Fig. 4).

Effect of PGE_2 and Forskolin on cAMP Production

One explanation for the greater inhibition by PGE_2 in presence of forskolin was that the dosage of neither effector alone maximized cAMP levels in IMR90 cells. To determine this possibility, cAMP production was measured in response to increasing PGE_2 concentration, as shown in Figure 5a. A half-maximal effect by PGE_2 took place at ~30nM. This effect was similar to that of PGE_2 on COL mRNA levels. The effect of PGE_2 on cAMP production began to plateau at 100 nM and reached a maximum at 1 μM. Cyclic AMP production was then measured in response to increasing concentrations of forskolin (Fig. 5b). Maximal cAMP production occurred in response to 100 μM forskolin. A stepwise increase in cAMP was detected between 2.5–25 μM forskolin. At 100 -250 μM, forskolin showed little further effect. Clearly, forskolin at 25 μM was suboptimal. The combined effect of 1

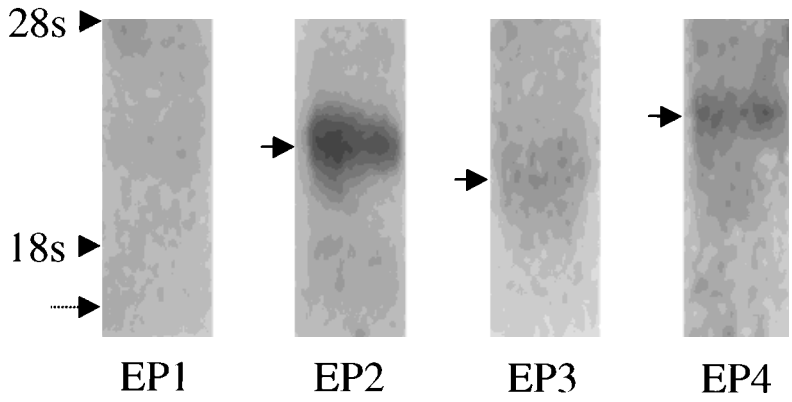

Fig. 2. Northern blot analyses of PGE_2 EP1, EP2, EP3, and EP4 receptor subtypes in IMR90 cells. Northern blot analysis was performed on 10 μg of total RNA isolated from confluent, quiescent, untreated IMR90 cells. Each lane was probed with specific subtype cDNA. The positions of the 28s and 18s rRNAs are marked with solid arrowheads. The hybridizing transcripts of EP2, EP3, and EP4 are marked with solid-lined arrows (→). The dotted-line arrow (· · ·→) represents the expected 1.6-kb EP1 hybridizing transcript as reported by Funk et al. [1993] and Fedyk and Phipps [1996] in human erythroleukemia cells. Autoradiography was performed at −80°C. The radiogram is a representative of results obtained from three separate experiments.

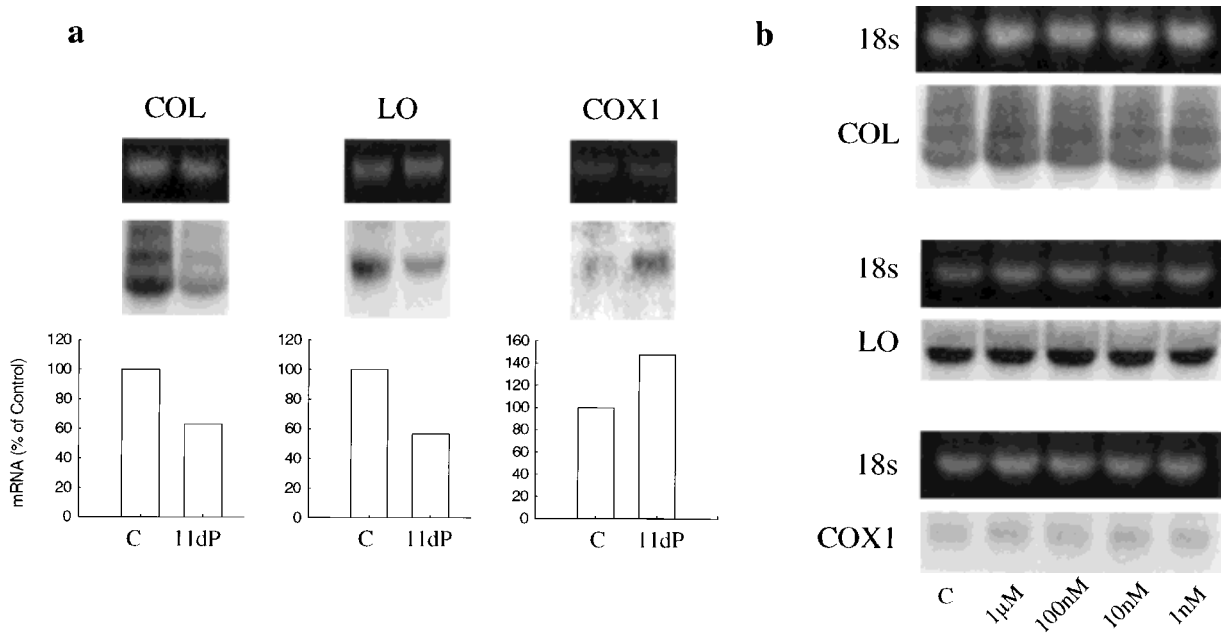

Fig. 3. Effect of EP2/EP4 agonist, 11-deoxy PGE_1 or EP3/EP1 agonist, sulprostone, on COL, LO, and COX1 mRNA levels. (**a**) IMR90 cells were incubated with or without 11-deoxy PGE_1 (1 μM) for 18 h in MEM containing 0.5% FBS. Total RNA was then isolated as described in Materials and Methods. RNA from two separate incubations of the same condition was then combined and hybridized with either COL, LO or COX1 cDNA. Each blot is representative of at least three separate experiments. 18s rRNA represents loading control. C = control (untreated), 11dP = 11-deoxy PGE_1. (**b**) IMR90 cells were treated as in (a). The cells were incubated with or without sulprostone at (1 nM–1 μM) for 18 h. The isolated total RNA represents two separate incubations in 100 × 20 mm culture plates with the same condition. The RNAs were probed with COL, LO or COX1 cDNA. C = control (untreated), concentrations at 1 μM–1 nM = sulprostone concentrations.

μM PGE_2 and 25 μM forskolin maximized the total cAMP production in a very similar manner as their combined reduction of COL mRNA shown in Figure 4.

Effect of 11-deoxy PGE_1 on TGF-β Induced COL mRNA and IL-1β Induced LO mRNA

The importance of the EP2/EP4 receptors in the regulation of COL and LO mRNA expression was further tested with the cytokines, TGF-β and IL-1β. As illustrated in Figure 6a, TGF-β increased COL mRNA levels by ~50%. IL-1β increased LO mRNA level over twofold above control (Fig. 6b). Treatment of IMR90 with either 11-deoxy PGE_1 or PGE_2 counteracted the effect by TGF-β and IL-1β. Both PGE_2 and 11-deoxy PGE_1 reduced TGF-β increased COL mRNA below that of the unstimulated control (Fig. 6a). Similarly, either 11-deoxy PGE_1 or PGE_2 reduced the IL-1β increased LO mRNA to that of the unstimulated control (Fig. 6b).

PGE_2 Receptor mRNA in Rat-1 Cells

Unlike the IMR90, Rat-1 cells do not express detectable EP2 receptor mRNA (see Fig. 7). In fact, of the four PGE_2 receptor types, only EP4 mRNA was detected; no EP3 or EP1 mRNA was observed. The Rat-1 cells are active producers of collagen [Granot et al., 1993]. These observations in sum presented an opportunity to explore the effect of PGE_2 on COL mRNA expression in the absence of an EP2 receptor.

Effect of PGE_2 and Forskolin on COL mRNA Production in Rat-1 Cells

To test the role of PGE_2 and cAMP in the regulation of COL mRNA steady-state levels, Rat-1 cells were incubated with PGE_2 (1 μM) or forskolin (25 μM) and probed for COL mRNA. As illustrated in Figure 8a, PGE_2 had only a slight effect on the amount COL mRNA expressed. However, forskolin (25 μM) increased COL mRNA markedly.

Effect of PGE_2, Forskolin, and 11-deoxy PGE_1 on cAMP Production in Rat-l Cells

The effect of PGE_2 or forskolin on cAMP production by Rat-1 cells is shown in Figure 8b.

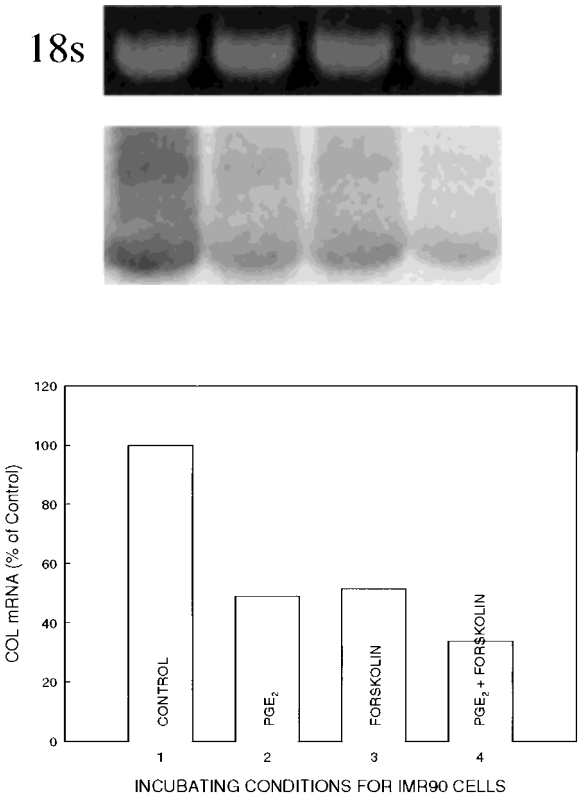

Fig. 4. Effect of PGE_2 and/or forskolin on COL mRNA level in IMR90 cells. IMR90 cells were made quiescent as described in Materials and Methods. The cells were then treated with PGE_2 (1 μM) and/or forskolin (25 μM) for 18 h. Total RNA was isolated as described in Materials and Methods and hybridized with COL cDNA probe. Northern blot analysis is a representative of three separate experiments. 18s rRNA represents loading control. Control = untreated.

These results confirm the lack of effect by PGE_2 on Rat-1 COL mRNA levels. PGE_2 had no effect on cAMP production by Rat-1 cells. In contrast, forskolin had a pronounced effect on cAMP production.

DISCUSSION

Our results illustrate that human embryonic lung fibroblasts, IMR90, express EP2, EP3, and EP4, but no detectable EP1 receptor mRNA. Among these, the EP2 receptor mRNA is predominant, whereas EP3 and EP4 mRNA are expressed minimally. We used sulprostone as an agonist for EP3/EP1 and 11-deoxy PGE_1 as an agonist for EP2/EP4, to determine whether the EP receptor subtypes were functionally different in regulating the expression of COL, LO, or COX1 mRNA levels. Sulprostone showed little effect on the three genes. However, 11-deoxy PGE_1 showed very similar potency to PGE_2 in limiting COL and LO and increasing COX1 mRNA levels. In addition, 11-deoxy PGE_1 ablated the TGF-β and IL-1β effected increases in COL and LO mRNA levels, respectively. This illustrates that the EP2 and possibly EP4 receptors are key mediators in the regulation of COL, LO and COX1 gene expression in IMR90 cells.

We examined the effects of forskolin, an activator of adenylate cyclase, on COL mRNA levels. Forskolin not only limited basal COL mRNA level, comparable to the effect of PGE_2, but also inhibited the TGF-β stimulated COL mRNA level (data not shown). Interestingly, a combination of forskolin and PGE_2 limited COL mRNA production to an even greater degree. It is possible that this synergy is due only to the suboptimal cAMP production by either agent alone. Another possibility is that the variation in maximal cAMP levels produced by PGE_2 and forskolin, and the variation in the level of negative regulation by these two effectors is due to cAMP compartmentalization. Such compartmentalization has been reported by Aass et al. (1988) and Jurevicius and Fischmeister (1996). Evidence for this compartmentalization is further supported by increasing number of adenylate cyclase and cAMP phosphodiesterase isoforms that differ in their spatial and temporal characteristics within a cell [Houslay and Milligan, 1997]. These differences may provide for the dynamic role of cAMP in various signaling cascades. To date, nine isoforms of adenylate cyclases and ~30 isoforms of cAMP phosphodiesterases have been reported in mammalian cells [Houslay and Milligan, 1997].

Recent publications indicate that EP4 and EP2 both increase intracellular cAMP [de Brum-Fernandes et al., 1996;Kiriyama et al., 1997] and bind similarly at high affinity with PGE_1, PGE_2, 11-deoxy PGE_1, and 16, 16-dimethyl-PGE_2 [Kiriyama et al., 1997]. Therefore, it is possible that in IMR90, both EP2 and EP4 receptors are active in the mechanism limiting the production and deposition of collagen. However, there are some important structural and functional differences between these two receptors. For example, in the cloned mouse EP4 receptors, there are ~3.5-fold more serine and threonine residues in the C-terminal than in the mouse EP2 receptors [Nishigaki et al., 1996]. Also, EP4 contains four potential PKA phosphor-

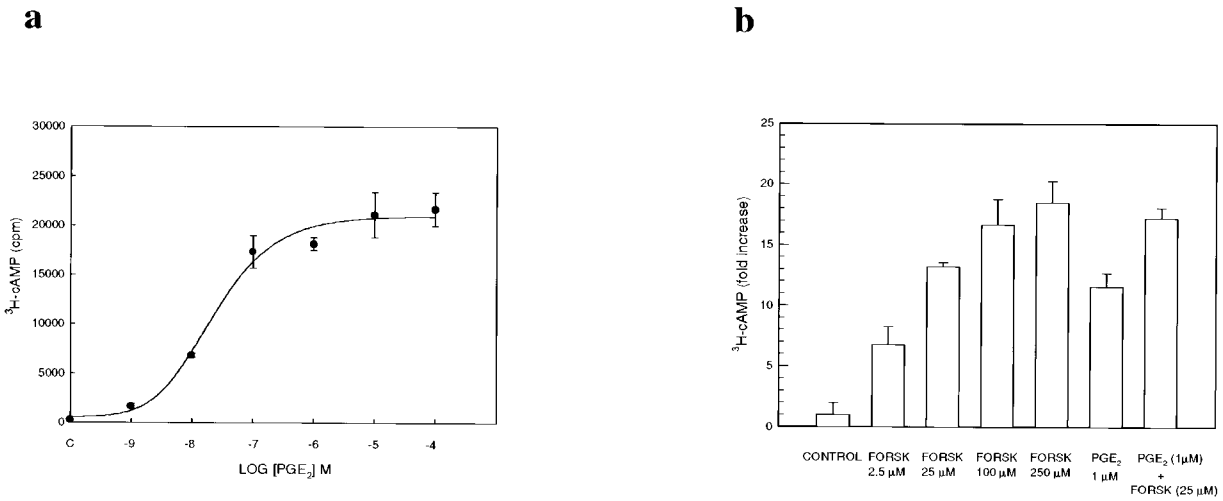

Fig. 5. Stimulation of cAMP production in IMR90 cells by PGE_2 and/or forskolin. (**a**) IMR90 cells were made quiescent and labeled with ^{3}H-adenine as described in Materials and Methods. The cells were treated with IBMX and with or without varying concentrations of PGE_2 (1 nM–100 μM) for 30 min. ^{3}H-cAMP was eluted as described in Materials and Methods and quantitated using liquid scintillation spectrometry. C = control (untreated). (**b**) IMR90 cells were treated and labeled with ^{3}H-adenine as in (a). The cells were treated with IBMX and with varying concentrations of forskolin (2.5–250 μM) or PGE_2 (1 μM) with or without forskolin (25 M) for 30 min. 3H-cAMP was eluted and quantitated as in (a). Values for both experiments are the means ± standard error of three experiments.

ylation sites, whereas none has been found in EP2 [Nishigaki et al., 1996]. Such phosphorylation sites may be critical in receptor desensitization [Liggett et al., 1993] and subsequently, the termination of signal transduction. Nishigaki et al. [1996] demonstrated a difference in PGE_2 induced-desensitization between EP4 and EP2 in CHO cells. In this experiment, PGE_2 induced EP4 receptors to undergo rapid desensitization but had no such effect on EP2. Importantly, EP2 generated a higher adenylate cyclase activity than EP4 in a time- dependent manner. Also, EP2 proved more responsive to 15-keto-PGE_2, a PGE_2 metabolite, than EP4 in stimulating adenylate cyclase. Interestingly, 15-keto-PGE_2 is metabolized by 15-hydroxy PG dehydrogenase, which is an enzyme that is abundant in the lung [Anggard et al., 1971]. Hence, EP2 mRNA, being the predominately expressed gene, and more capable in producing cAMP than EP4, is most likely responsible for the PGE_2 effect on COL and LO mRNA levels in IMR-90.

Rat-1 cells are active producers of the COL peptide [Granot et al., 1993]. Examination of PGE_2 receptor subtypes in these cells shows that they express EP4 mRNA but lack detectable EP1, EP2, and EP3 mRNA. We treated these cells with PGE_2 or forskolin to examine whether either effected COL mRNA and cAMP levels. We found that PGE_2 had little effect on COL mRNA levels in these cells. However, forskolin upregulated the expression of COL mRNA. Furthermore, PGE_2 had no effect on cAMP level with 30 min incubation, whereas forskolin increased it by 10-fold. Interestingly in the Rat-1 cells, EP4 receptors are predominant. These results further suggest that EP4 is a poor inducer of cAMP production, and after 30 min incubation its effect on cAMP level is not detectable. These results strengthen the conclusion that the EP2 receptors are interacting with PGE_2 to effect the COL, LO and COX1 gene expression.

The effect of cAMP in Rat-1 cells appears to be opposite to that found in IMR90 in which forskolin depresses COL mRNA levels. Interestingly, previous studies using MC3T3-E1, a mouse osteoblast-like cell line, showed that forskolin, which increases cAMP levels, increases COL synthesis [Hakeda et al., 1987]. Yet, an elevation of cAMP in UMR 106–06 rat osteosarcoma cells was shown to decrease collagen synthesis [Pun, 1989; Iida-Klein et al., 1992]. Understanding of these apparently contradictory effects of cAMP clearly requires further investigation and may ultimately provide important strategy in the regulation of collagen synthesis and deposition in response to injury and in fibrotic disease.

It appears that limitation by PGE_2 of collagen production and its deposition as an extracel-

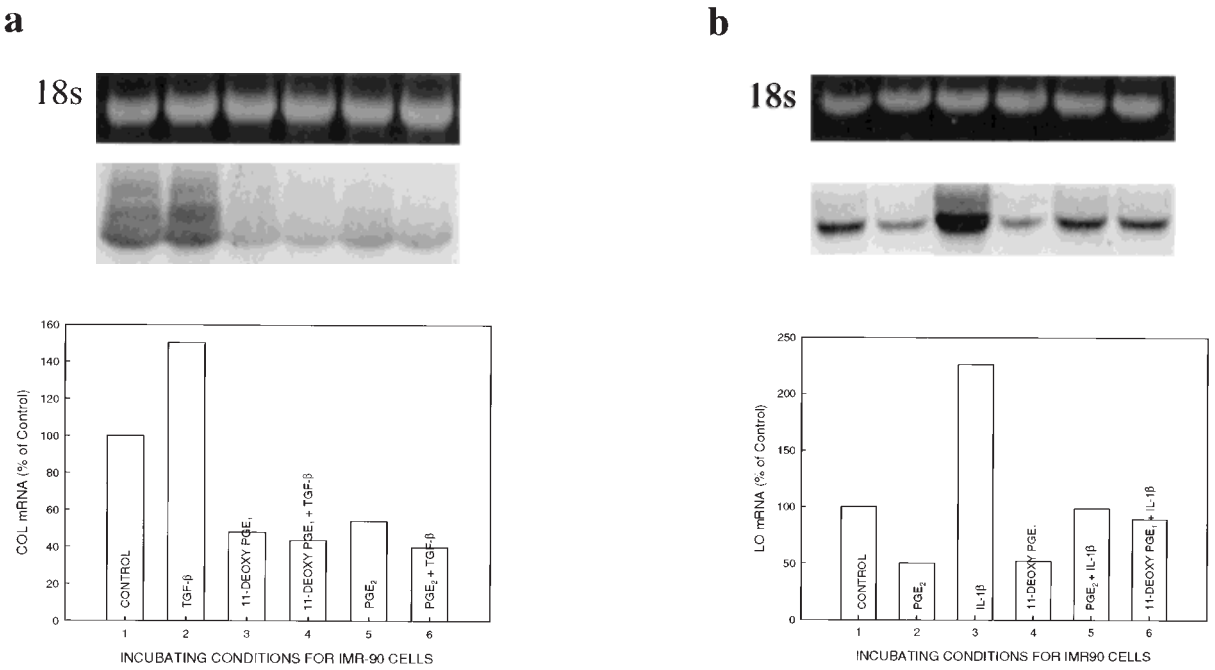

Fig. 6. Effect of PGE_2 or 11-deoxy PGE_1 on TGFβ stimulated COL mRNA and IL-1β stimulated LO mRNA levels in IMR90 cells. (**a**) IMR90 cells were grown to confluence and made quiescent as described in Materials and Methods. The cells were untreated (control) or treated with PGE_2 (1 μM) or 11-deoxy PGE_1 (1 μM) with or without TGFβ (100 ng/ml) for 18 h. Total RNA isolated from two separately treated 100 × 20 mm culture plates was combined; 10 μg of total RNA was fractionated by electrophoresis and transferred to nylon membrane as described in Materials and Methods. Ten μg of the total RNA was hybridized with COL cDNA. (**b**) IMR90 cells were grown to confluence and made quiescent as described in Materials and Methods. The cells were untreated (control) or treated with PGE_2 (1 μM) or 11-deoxy PGE_1 (1 μM) with or without IL-1β (100 pg/ml) for 18 h. The total RNA was isolated as in (a) and hybridized with LO cDNA. For both (a) and (b), 18s rRNA represents loading control.

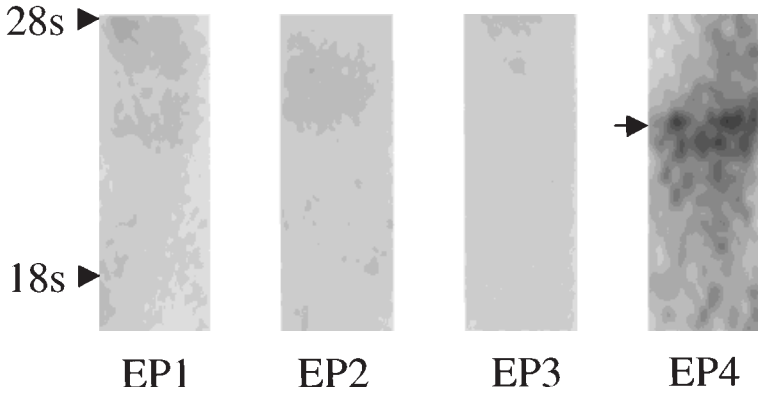

Fig. 7. Northern blot analyses representing PGE2 EP1, EP2, EP3, and EP4 receptor subtypes in Rat-1 cells. Northern blot analysis was performed on 10 μg of total RNA isolated from confluent, untreated Rat-1 cells. Each lane was probed with specific subtype cDNA. The positions of the 28s and 18s rRNAs are marked with solid arrowheads. The hybridizing transcript of EP4 are marked with solid-lined arrows (→). No detectable hybridization occurred for EP1, EP2, or EP3. Autoradiography was performed at −80°C. The radiogram is a representative of results obtained from two separate experiments.

lular matrix involves the EP2 receptor with cAMP at the core of this signal transducing mechanism. These results may be pertinent to mechanisms related to tissue scarring or connective tissue-linked diseases. In fact, the limiting by PGE_2 of cytokine stimulated collagen deposition and the failure or reversal of this mechanism could prove critical to the fibrotic sequel of inflammation and certain connective tissue diseases.

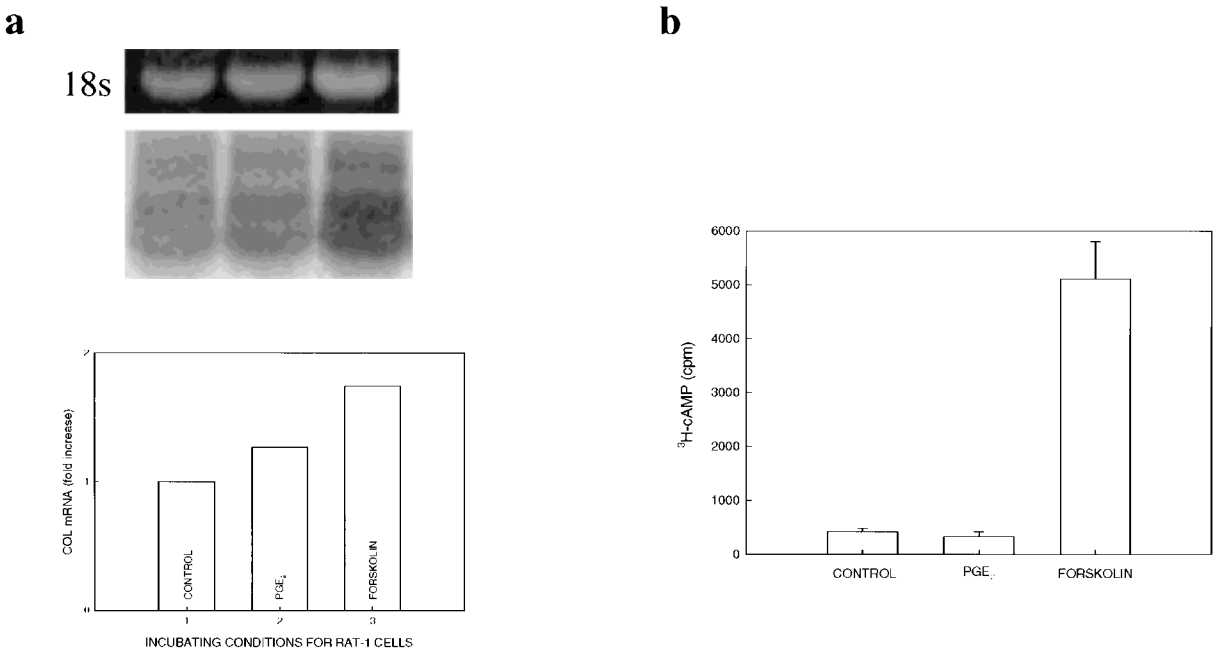

Fig. 8. Effect of PGE_2 or forskolin on Rat-1 cells. (**a**) Rat-1 cells grown to confluence were treated with PGE_2 (1 μM) or forskolin (25 μM) for 18 h. Total RNA was isolated of which 10 μg was used to hybridize with COL cDNA probe as described in Materials and Methods section. The northern blot analysis is a representative of two separate experiments; 18s rRNA represents loading control. (**b**) Rat-1 cells were labeled with ^{3}H-adenine as described in Materials and Methods. The cells were then treated with or without PGE_2 or forskolin for 30 min. ^{3}H-cAMP was eluted and quantitated as described in Materials and Methods section. Values are the means ± standard error of three experiments.

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