Regulation of Expression of Matrix Metalloproteinase-9 in Early Human T Cells of the HSB.2 Cultured Line by the EP3 Subtype of Prostaglandin E2 Receptor*

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Prostaglandin E2 (PGE2) is a product of the cyclooxygenation of arachidonic acid released from cellular phospholipids that potently mediates many biological functions in the cardiovascular, pulmonary, renal, endocrine, gastrointestinal, neural, reproductive, and immune systems (1, 2). Cell surface expression of multiple functionally distinct subtypes of PGE2 receptors (Rs) is a principal determinant of the diversity and specificity of cellular effects of PGE2. PGE2 is recognized and transduces cellular effects specifically by interacting with PGE2 Rs of at least four subtypes, designated the EP1, EP2, EP3, and EP4 Rs. These subtypes of PGE2 Rs differ in structure, ligand-binding properties, tissue distribution, and coupling to signal transduction pathways (2). All subtypes of PGE2 Rs have recently been cloned and shown to be members of the G protein-coupled seven-transmembrane domain superfamily (1, 2). EP3 Rs mediate increases in the intracellular concentration of calcium ([Ca2+]i) (3). EP2 and EP4 Rs activate adenylyl cyclase via Gs and stimulate increases in the intracellular concentration of cAMP ([cAMP]i) (4–6). Multiple isoforms of EP2 Rs not only inhibit adenylyl cyclase, resulting in a decrease in [cAMP], elevated by forskolin or other agonists via Gs, but also stimulate increases in [Ca2+]i via Gq/G11 or G12 (1, 7–9). Certain isoforms of the EP3 R in nonhuman species also activate adenylyl cyclase via Gs and transduce increases in [cAMP], (8, 9).

PGE2 potently mediates and modulates cellular and humoral immune responses by stimulating or inhibiting the functions of many different types of immune cells (10). At physiological concentrations, PGE2 enhances elements of macrophage differentiation but inhibits functional activation and enhances B cell production of IgG1 and IgE while inhibiting that of IgM (11). Of central importance in most host defense and autoimmune responses is that PGE2 inhibits T cell proliferation, differentiation, expression of membrane Rs, secretion of diverse cytokines, cytotoxicity, and other specific effector functions in cellular immune reactions (10). Some PGE2 effects on T cells appear to be subset-selective, as for stimulation of the proliferative responses of a suppressor subset of T cells and concurrent suppression of the responses of a subset of helper T cells (10). PGE2 effects on T cells have been attributed almost exclusively to increases in [cAMP], which are transduced by EP2 and/or EP4 Rs. Expression of the EP3 subtype of PGE2 Rs by T cells at a level capable of altering T cell functions has not been described nor have the functional consequences of EP3, R-mediated signaling of T cells. The cultured line of human leukemic T cells, termed HSB.2, is an early “double negative” thymocyte

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bearing CD2 and CD7 but not CD3, CD4, or CD8 (12, 13). It is now shown that HSBCells express predominantly the EP3 subtype of PGE2 R and that PGE2 stimulates increases in HSBCellular content and secretion of matrix metalloproteinase (MMP)-9 by EP3 R-mediated and [Ca2+]i-dependent enhancement of transcription of mRNA encoding MMP-9.

EXPERIMENTAL PROCEDURES

Materials—[5,6,8,11,12,14,15-3H]PGE2 (153 Ci/mmol, DuPont NEN, M&B 28767 (Rhone-Poulenc Rorer Research, Dagenham, Essex), sulprostone (Schering Pharmaceuticals, Berlin), PGE2, PGD2, PGE2α, PGD2α, PGI2 (Upjohn Co., Kalamazoo, MI), SC 19220 and misoprostol (Searle, Skokie, IL), cAMP radioimmunooassay kit (DuPont NEN), mouse monoclonal antibody specific for human PGE2 R, MMP-9 (Oncogene Science, Cambridge, MA), 3-isobutyl-1-methylxanthine, 9-tetrahydro-2-furyl-adenosine (SQ 22536), H-89 and KT5720 (Calbiochem), fura-2 acetoxymethylester (Molecular Probes, Inc. Eugene, OR), genistein and tyrphostin (Life Technologies, Inc.), ovalbumin, cycloheximide, actinomycin D, type A porcine skin-derived gelatin, thapsigargin, ionomycin, forskolin, dibutyryl-cAMP, and pertussis toxin (Sigma) were obtained from the designated sources.

Cultures of HSBCells—Human leukemic T cells of the HSBCells line (13)(obtained from American Type Culture Collection) were cultured in RPMI 1640 medium (University of California, San Francisco, Cell Culture Facility) with 15 mM HEPES, 10% (v/v) fetal bovine serum (HyClone Laboratories, Inc., Logan, UT), 100 units/ml of penicillin and 100 μg/ml of streptomycin in a complete RPMI medium. Cultures were maintained at 37°C in a humidified atmosphere of 5% CO2/95% air at a 95% relative humidity. HSB.2 cells were washed two times with Ca2+- and Mg2+-free HBSS with 1 mM EGTA at a concentration of 106 HSB.2 cells/ml. Poly(A) RNA was isolated from HSBCells with TRIzol Reagent kit (Life Technologies, Inc.). First strand cDNAs were synthesized from HSBCellular total RNA with random hexamer primers and Superscript II reverse transcriptase (Life Technologies, Inc.) and were used as templates for polymerase chain reaction with 36 cycles of 95°C for 1 min, 55°C for 1 min, and 72°C for 1 min. The MMP-9 primers were 5'-AGACCTGAGGAACCAATCT (upstream) and 5'-GGCACCTGAGGAATGCTA (downstream) (16). The resultant reverse transcription-polymerase chain reaction products were analyzed by electrophoresis in a 2% agarose gel, and the product of 1.1 kilobase pairs of MMP-9 cDNA was extracted and purified from agarose gels and 32P-labeled for Northern blot analyses.

Replicate pellets of 106 HSBCells were washed and incubated in 20 ml of protein-free Iscove's/RPMI 1640 medium (11, 13) at 37°C for 24 h. 10−9 M PGE2 or sulprostone was added to HSBCells in incubation at 24, 12, 4, and 1 h before harvesting the cells. Poly(A) RNA was isolated from the control and treated HSBCells by the Fast Track Kit (Invitrogen, San Diego, CA) and poly(A) RNA was then in 50 mM Tris-HCl/50 mM NaCl/5 mM CaCl2 for 24 h at 37°C to permit digestion of gelatin by MMPs. After staining undigested protein with Coomassie Blue, the decrease in staining of each band that reflected protease activity was determined by densitometry with a ScanJet IIIC and quantified by NIH Image 1.41 software. All results were expressed as relative percentages of untreated controls.

Replicate 5-μg protein aliquots of the secreted and cytosolic supernatants were subjected to 10% SDS-polyacrylamide gel electrophoresis for quantification by immunoreactivity, and the proteins resolved were then transferred by electroblotting to a 0.45-μm pore nitrocellulose membrane (Hybond, Amersham Corp.). The blots were developed with 1 μg/ml of mouse monoclonal IgG antibodies specific for human MMP-9, MMP-2, MMP-1, and MMP-3, and then in a 1/200 dilution of horseradish peroxidase-labeled sheep anti-mouse IgG (Amersham Corp.). Luminescence analysis was performed according to the manufacturer's protocol (ECL, Amersham Corp.), and the MMP-9 was quantified by densitometry.

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misoprostol (EP$_2$/EP$_3$/EP$_3$ R agonist), and butaprost (EP$_2$ R-selective agonist) concentration-dependently stimulated increases in [cAMP], in HSB.2 cells, whereas the EP$_2$/EP$_3$ R-selective agonist sulprostone did not affect the level of [cAMP] (Fig. 2). Thus the EP$_2$ Rs and EP$_3$ Rs detected by binding of [H]PGE$_2$ are coupled to adenylyl cyclase in HSB.2 cells as expected. PGE$_2$ and the EP$_3$/EP$_1$ R-selective agonist sulprostone also concentration-dependently stimulated increases in [Ca$^{2+}$]$_i$ (Table I). The EP$_2$ R-selective antagonist SC-19220 at 30 nM, which itself did not alter [Ca$^{2+}$]$_i$, had no effect on increases in [Ca$^{2+}$]$_i$, evoked by 10$^{-7}$ and 10$^{-5}$ M sulprostone (Table I), suggesting that the [Ca$^{2+}$]$_i$ elevation induced by sulprostone was mediated solely by an EP$_2$ R-dependent mechanism. This finding further confirmed the presence of EP$_2$ Rs and the absence of EP$_3$ Rs in HSB.2 cells as suggested by the binding data. As expected, 10$^{-6}$ M butaprost did not elicit increases in [Ca$^{2+}$]$_i$ in HSB.2 cells (Table I). Furthermore, the elevations of [Ca$^{2+}$]$_i$, in response to PGE$_2$ and sulprostone were not changed by elimination of extracellular Ca$^{2+}$ (18–20), elevated the basal level of [Ca$^{2+}$]$_i$, but suppressed the increases of [Ca$^{2+}$]$_i$, in response to 10$^{-7}$ M PGE$_2$ (Table I). These results suggest that PGE$_2$-elicited increases in [Ca$^{2+}$]$_i$, are attributable to release of Ca$^{2+}$ from intracellular stores linked to EP$_2$ R signals.

Enhancement by PGE$_2$ of the Cytosolic Level and Secretion of MMP-9 by HSB.2 Cells—Gelatin zymographic analyses of the secretion and extracts of HSB.2 cells revealed one predominantly gelatinolytic activity of 92 kDa (Fig. 3, A and B) that corresponds to the molecular mass of MMP-9 (21). Neither the related MMP-2 (72 kDa) gelatinase nor other MMPs, such as MMP-1 and MMP-3 (55–59 kDa), were detected. Secreted MMP-9 activity was detected as early as 4 h, reached a maximal level at 24 h, and persisted through 48 h (data not shown). At 24 h, 10$^{-9}$, 10$^{-8}$, 10$^{-7}$, and 10$^{-6}$ M PGE$_2$ increased MMP-9 activities in a concentration-dependent manner ranging from means of 1.1–3.0-fold higher than unstimulated control cells (Fig. 3A). Cytosolic MMP-9 activities also were augmented by PGE$_2$ (Fig. 3B) with a pattern similar to that of the secreted MMP-9, suggesting that PGE$_2$ increases both the synthesis and secretion of MMP-9 protein. Western blot analyses of the secreted (Fig. 3C) and cytosolic (data not shown) MMP from HSB.2 cells confirmed the identity of MMP-9 and the stimulatory effect of PGE$_2$ on the level of MMP-9 protein. When the same amount of total protein was used for each Western blot analysis, the amount of MMP-9 immunoreactive protein was increased by PGE$_2$ dose-dependently, as compared with the control in buffer alone (Fig. 3C), implying that PGE$_2$ stimulated the levels of MMP-9 activity in part by increasing the cytosolic and secreted amounts of MMP-9 (Fig. 3, A and B). Moreover, indomethacin at 10$^{-5}$ M, which suppresses endogenous PGE$_2$ synthesis, did not change the basal MMP-9 level, suggesting either that HSB.2 cells do not produce relevant amounts of PGE$_2$ or that the basal MMP-9 activity of HSB.2 cells is independent of endogenous PGE$_2$.

Mediation of PGE$_2$ Effect on MMP-9 by an EP$_3$ R-dependent Mechanism Involving Increases in [Ca$^{2+}$]$_i$, from Intracellular Stores—PGE$_3$ and the EP$_3$ R-directed agonists sulprostone, M&B 28767, and misoprostol enhanced MMP-9 activity of HSB.2 cells to the same extent as PGE$_2$ or higher at optimal concentrations, whereas there was no effect with the other synthetic prostanoids PGF$_{2\alpha}$, PGD$_2$, and PGL$_2$ nor the EP$_2$ R-selective agonist butaprost (Fig. 4A). The dependence of MMP-9 enhancement on EP$_3$ R-directed effects of both natural and pharmacological agonists confirms the specificity of coupling of MMP-9 responses to the EP$_3$ R subtype. Neither dibutyryl-cAMP nor the adenyl cyclase stimulator forskolin or inhibitor SQ 22536 changed significantly the activity of MMP-9 (data not shown), diminishing the possibility that the effect of PGE$_2$ is through a cAMP-dependent mechanism.

When HSB.2 cells were washed and incubated with protein- and Ca$^{2+}$-free medium containing 1 mM EGTA, we observed that HSB.2 cell growth was inhibited by 62% (mean ± S.E., n = 3) after 24 h when compared with that in medium containing 1 mM Ca$^{2+}$. Consistently, the basal and PGE$_2$-stimulated levels of MMP-9 detected in Ca$^{2+}$-free medium were

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**Fig. 1.** Specific binding of [H]PGE$_2$ to HSB.2 cells. Competitive inhibition of the binding of [H]PGE$_2$ by nonradioactive prostanoids (A) and synthetic prostanoid analogues (B). Each point represents the mean ± S.E. (n = 9). The mean IC$_{50}$ values derived from analysis by the LIGAND program were 3.4 ± 0.7 nM for PGE$_2$, 397 ± 14 nM for PGF$_{2\alpha}$, 486 ± 76 nM for PGD$_2$, and > 10 μM for PGI$_2$.

**Fig. 2.** EP$_2$ R- and EP$_3$ R-dependent increases on [cAMP], of HSB.2 cells. Each point represents the mean ± S.E. of the results from three experiments performed with triplicate samples. PGE$_2$, misoprostol, and butaprost stimulated increases in [cAMP], in HSB.2 cells with respective maxima of 44.4 ± 5.0-, 33.6 ± 5.0-, and 9.8 ± 1.5-fold (mean ± S.E., n = 9) at 10$^{-6}$ M for each agonist.

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Mediation of PGE$_2$ Effect on MMP-9 by an EP$_3$ R-dependent Mechanism Involving Increases in [Ca$^{2+}$]$_i$, from Intracellular Stores—PGE$_3$ and the EP$_3$ R-directed agonists sulprostone, M&B 28767, and misoprostol enhanced MMP-9 activity of HSB.2 cells to the same extent as PGE$_2$ or higher at optimal concentrations, whereas there was no effect with the other synthetic prostanoids PGF$_{2\alpha}$, PGD$_2$, and PGL$_2$ nor the EP$_2$ R-selective agonist butaprost (Fig. 4A). The dependence of MMP-9 enhancement on EP$_3$ R-directed effects of both natural and pharmacological agonists confirms the specificity of coupling of MMP-9 responses to the EP$_3$ R subtype. Neither dibutyryl-cAMP nor the adenyl cyclase stimulator forskolin or inhibitor SQ 22536 changed significantly the activity of MMP-9 (data not shown), diminishing the possibility that the effect of PGE$_2$ is through a cAMP-dependent mechanism.

When HSB.2 cells were washed and incubated with protein- and Ca$^{2+}$-free medium containing 1 mM EGTA, we observed that HSB.2 cell growth was inhibited by 62% (mean ± S.E., n = 3) after 24 h when compared with that in medium containing 1 mM Ca$^{2+}$. Consistently, the basal and PGE$_2$-stimulated levels of MMP-9 detected in Ca$^{2+}$-free medium were
MMP-9 is independent of extracellular Ca\textsuperscript{2+} cell expression of MMP-9 by HSB.2 cells in Ca\textsuperscript{2+}

PGE\textsubscript{2} still significantly stimulated MMP-9 secretion in Ca\textsuperscript{2+}free medium when compared with the control level without PGE\textsubscript{2} (Fig. 4). However, PGE\textsubscript{2} significantly stimulated MMP-9 secretion in Ca\textsuperscript{2+}-free medium when compared with the control level without PGE\textsubscript{2} (Fig. 4B), suggesting that PGE\textsubscript{2} enhancement of secretion of MMP-9 is independent of extracellular Ca\textsuperscript{2+}. Western blots also showed that PGE\textsubscript{2} dose-dependently increased intracellular expression of MMP-9 by HSB.2 cells in Ca\textsuperscript{2+}-free medium (data not shown). Ionomycin, an ionophore that increases the permeability of plasma membrane to divalent cations, such as Ca\textsuperscript{2+}, evoked increases in [Ca\textsuperscript{2+}], of HSB.2 cells (in buffer with 1 mM Ca\textsuperscript{2+}) that attained mean maxima (n = 4) of 1.1-, 6-, and 100-fold at respective concentrations of 10\textsuperscript{6}, 10\textsuperscript{-7}, and 10\textsuperscript{-9} M. However, when HSB.2 cells were incubated with 10\textsuperscript{-9} M ionomycin in medium containing 1 mM Ca\textsuperscript{2+}, neither basal nor PGE\textsubscript{2}-stimulated increases in MMP-9 secretion was significantly affected (data not shown), ruling out the possibility that increases in Ca\textsuperscript{2+} influx mediate the effect of PGE\textsubscript{2} on MMP-9.

To investigate further whether EP\textsubscript{3} R-dependent increases in cytosolic and secreted MMP-9 are mediated by increases in [Ca\textsuperscript{2+}], we preincubated HSB.2 cells with 10\textsuperscript{-8} M atropine, respectively. Zymographic analyses were performed with the same amount of secreted proteins. A, EP\textsubscript{3} R-mediated effect. Supernatant protein samples in lanes 1–9 are from HSB.2 cells incubated for 24 h with buffer alone or with 10\textsuperscript{-9} M PGE\textsubscript{2}, PGE\textsubscript{1}, PGI\textsubscript{2}, PGD\textsubscript{2}, sulprostone, or butaprost, respectively. B, PGE\textsubscript{2} effect on MMP-9 is independent of extracellular Ca\textsuperscript{2+}. The samples in lanes 1–5 are: buffer control, 10\textsuperscript{-6}, 10\textsuperscript{-7}, and 10\textsuperscript{-8} M PGE\textsubscript{2} after 24 h in Ca\textsuperscript{2+}-free medium. The results show that 10\textsuperscript{-6}, 10\textsuperscript{-7}, and 10\textsuperscript{-8} M PGE\textsubscript{2} significantly stimulated MMP-9 secretion with respective increases of 3.0 ± 0.4, 5.4 ± 0.4, and 5.4 ± 0.6-fold (mean ± S.E., n = 4, p < 0.05 by Student’s t test), as determined by densitometry, when compared with the control level without PGE\textsubscript{2}. C, PGE\textsubscript{2} effect on MMP-9 requires Ca\textsuperscript{2+} release from intracellular stores. The samples are: buffer control (lane 1), 10\textsuperscript{-6} M PGE\textsubscript{2} (lane 2), 10\textsuperscript{-7}, and 10\textsuperscript{-8} M thapsigargin alone (lanes 3–5, respectively), and 10\textsuperscript{-7} M thapsigargin together with 10\textsuperscript{-8} M PGE\textsubscript{2} (lanes 6–8, respectively) after 24 h. The results presented in panels A, B, and C are representative of those from three or four experiments.

**TABLE I**

**Induction by PGE\textsubscript{2} and sulprostone of increases in [Ca\textsuperscript{2+}], in HSB.2 cells**

| Agonist          | Concentration | [Ca\textsuperscript{2+}], % |
|------------------|---------------|------------------------------|
|                  | Medium with Ca\textsuperscript{2+} | Medium without Ca\textsuperscript{2+} |
| PGE\textsubscript{2} | 10\textsuperscript{-8} M |
| Sulprostone      | 10\textsuperscript{-8} M | 122 ± 14 | 175 ± 14 |
| SC19220          | 10\textsuperscript{-8} M | 182 ± 11 | 232 ± 37 |
| SC19220 (10\textsuperscript{-5} M) with sulprostone | 10\textsuperscript{-8} M | 298 ± 21 | 289 ± 48 |
| Thapsigargin     | 10\textsuperscript{-7} M | 146 ± 5 | 133 ± 5 |
| Subsequent PGE\textsubscript{2} | 10\textsuperscript{-7} M | 222 ± 16 | 193 ± 26 |
| Butaprost        | 10\textsuperscript{-7} M | 311 ± 44 | 275 ± 53 |

*Expressed as a percentage of the level elevated by 10\textsuperscript{-7} M thapsigargin pretreatment for 10 min.
PGE$_2$ Regulation of T Cell Matrix Metalloproteinase

The expression by T cells of multiple functionally distinct subtypes of PGE$_2$ Rs, designated EP$_1$, EP$_2$, EP$_3$, and EP$_4$ Rs, is a principal determinant of specificity and diversity of the immune effects of PGE$_2$ (1, 2). Regulation of T cell functions was not attributed previously to EP$_3$ Rs. We now show that the cultured line of human leukemic T cells, termed HSB.2, co-expresses a total of 7282 ± 1805 EP$_3$, EP$_4$, and EP$_2$ Rs per cell with a $K_d$ of 3.7 ± 1.4 nm. HSB.2 T cells differ from blood and lymphoid tissue T cells in expressing predominantly EP$_3$ Rs and lower levels of EP$_2$ Rs and EP$_4$ Rs. The EP$_2$/EP$_4$ R-selective agonist sulprostone and EP$_3$/EP$_4$ R-selective agonists M&B 28767 and misoprostol competitively inhibited the binding of [H]$^3$H$^3$H$^3$H$^3$PGE$_2$ to HSB.2 cells (Fig. 1). In contrast, the EP$_2$ R-selective agonist butaprost inhibited [H]$^3$H$^3$H$^3$H$^3$PGE$_2$ binding much less at only the highest concentration of 10$^{-6}$ M, and the EP$_3$ R-selective antagonist SC-19220 did not alter [H]$^3$H$^3$H$^3$H$^3$PGE$_2$ binding (Fig. 1). These results indicated predominant expression of EP$_2$ Rs, fewer EP$_4$ Rs, a much lower level of EP$_3$ Rs, and no detectable EP$_1$ Rs in HSB.2 cells.

Assessment of adenyl cyclase signaling revealed that PGE$_2$, misoprostol, and a higher concentration of butaprost evoked increases in [cAMP] in HSB.2 cells (Fig. 2), confirming expression of functional EP$_2$ Rs and EP$_4$ Rs. The isoforms of EP$_2$ Rs in some nonhuman species that transduce increases in [cAMP] (8, 9) were not detected in HSB.2 cells, because the EP$_3$ R-selective agonist sulprostone did not stimulate increases in [cAMP] (9). This is consistent with our previous finding that none of the human EP$_3$ Rs isoforms mediated an increase in [cAMP] (7). The increases in [Ca$^{2+}$]i by PGE$_2$ and the EP$_2$/EP$_4$ R-selective agonist sulprostone and the failure of a maximal concentration of the EP$_3$ R-selective antagonist SC-19220 to dampen increases in [Ca$^{2+}$]i, evoked by sulprostone (Table I) argue strongly against the presence of EP$_3$ Rs in HSB.2 cells and confirm the presence of functionally relevant EP$_3$ Rs. EP$_3$ Rs mediate increases in [Ca$^{2+}$]i by stimulating the entry of extracellular Ca$^{2+}$ (3); thus our finding that the elevations of [Ca$^{2+}$]i by PGE$_2$ and sulprostone were not changed by elimination of extracellular Ca$^{2+}$ further confirms the absence of EP$_1$ Rs in HSB.2 cells. EP$_2$ Rs are therefore the exclusive transducers of increases in [Ca$^{2+}$]i, evoked by PGE$_2$ in HSB.2 cells.

A family of MMPs is the principal physiological system that degrades diverse components of extracellular matrix (21). In human blood T cells and some human T lymphoblastoma cells, PGE$_2$ stimulates surface expression and secretion of MMPs-2, -3, and -9, which create channels in the basement membrane required to admit migrating blood T cells and Tsup-1 cells (15, 22). Using HSB.2 cells as a T cell model, we thus examined the possibility that EP$_3$ Rs mediate regulation of MMPs in T cells responding to PGE$_2$ and the biochemical signaling mechanisms by which EP$_3$ Rs transduce PGE$_2$-evoked increases in MMPs. We demonstrated that stimulation of MMP-9 in HSB.2 cells by PGE$_2$ is attributable to [Ca$^{2+}$]i-dependent EP$_3$ Rs R mediation of increases in message transcription. It has been reported that treatment of HSB.2 cells with the tumor promoter 12-O-tetradecanoylphorbol 13-acetate elicits secretion of MMP-9 activity (23). We now confirm by zymography and Western blots that MMP-9 is the exclusive MMP secreted constitutively by HSB.2 cells. Zymographical analyses revealed that the cytosolic level and secretion of MMP-9 were increased maximally after 24 h of incubation of HSB.2 cells with 10$^{-7}$–10$^{-6}$ M PGE$_2$. Parallel Western blot analyses showed that PGE$_2$ stimulated MMP-9 activity in part by increasing both MMP-9 protein expression and secretion (Fig. 3).

PGE$_2$, but not PGE$_1$, PGE$_4$, or PGE$_3$, enhanced MMP-9 activity of HSB.2 cells to the same extent as PGE$_2$, reflecting PGE$_2$ R specificity on MMP-9 and the similar preference of PGE$_2$ Rs for PGE$_2$ and PGE$_1$, which was observed in other immune cells.
and tyrphostin at concentrations of $10^{-7}$ to $10^{-4}$ M, or with protein kinase A/protein kinase G inhibitors H-89 and KT5720 at concentrations of $10^{-9}$ to $10^{-6}$ M did not change the basal and PGE2 elevated level of MMP-9 (data not shown). Further work is needed to elucidate biochemical signal transduction mechanisms by which PGE2 affects MMP-9 in HSB.2 T cells.

It has been demonstrated that MMP-1, -3, and -9 expression is regulated at the transcriptional level by factors including 12-O-tetradecanoylphorbol 13-acetate, tumor necrosis factor α, epidermal growth factor, platelet-derived growth factor, nerve growth factor, interleukin-1, transforming growth factor-β, progesterone, and corticosteroids (21, 22). We now show that PGE2 enhances MMP-9 expression by increasing transcription as well. Prevention of PGE2-induced increases in the cytosolic level and secretion of MMP-9 in HSB.2 cells by both protein synthesis inhibitor cycloheximide and RNA synthesis inhibitor actinomycin D suggested a requirement for de novo protein synthesis and transcription. Northern analyses revealed enhancement of the level of MMP-9 mRNA after 12–24 h of PGE2 treatment (Fig. 5). The ability of the EP3 R-directed agonist butaprost to mimic a PGE2 stimulatory effect on MMP-9 mRNA (Fig. 5) supports the hypothesis that EP3 Rs signal predominantly at the transcriptional level. Thus, stimulation of MMP-9 in HSB.2 T cells by PGE2 is attributable to [Ca2+] i-dependent EP3 R-mediation of increases in message transcription.

The HSB.2 T cell (12) is at an early thymocyte stage (13) and expresses predominantly EP2 and EP3 Rs, of which the former is the principal transducer of PGE2 effects on MMP-9. However, mature human blood CD4+ and CD8+ T cells express principally EP3 Rs and only the CD8+ subset bears a prominent number of EP3 Rs. In human blood T cells of mixed CD4+ and CD8+ composition, PGE2 stimulates surface expression and secretion of MMP-2 and -3, as well as MMP-9 (15), but the PGE2 R subtype dependence has not been defined. Whether HSB.2 cell is representative of human normal early thymocytes and whether the EP3 Rs of CD8+ T cells transduce increases in MMP-9 content and secretion remain to be evaluated in the corresponding population of T cells.

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