Cyclic Adenosine 3',5'-Monophosphate Suppresses Interleukin 1-induced Synthesis of Matrix Metalloproteinases but Not of Tissue Inhibitor of Metalloproteinases in Human Uterine Cervical Fibroblasts*

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Interleukin 1 (IL-1) mediates many cellular functions, but the signal transduction mechanisms of its actions are not clearly understood. Here, we have examined the exact participation of cAMP in the IL-1-induced production of the precursors of matrix metalloproteinase (MMPs) and their specific inhibitor, tissue inhibitor of metalloproteinases (TIMP) in human uterine cervical fibroblasts. IL-1 significantly augmented the production of proMMP-1 (vertebrate procollagenase), proMMP-3 (prostomelysin), and TIMP without detectable changes in the intracellular level of cAMP. Dibutyryl cAMP (Bt2cAMP) and the cAMP elevating agent (forskolin) did not replace IL-1 as MMP inducers. On the contrary, the IL-1-mediated induction of proMMP-1 and proMMP-3 was significantly suppressed by treatment of the cells with Bt2cAMP, forskolin, or theophylline. The suppressive effect of Bt2cAMP on the IL-1-induced production of proMMP-1 and -3 was not due to the inhibition of zymogen secretion, but resulted from the decrease in the steady-state levels of proMMP-1 and proMMP-3 mRNAs. In contrast, Bt2cAMP slightly enhanced the IL-1-induced production of TIMP. The synthesis of proMMP-2 (72-kDa procollagenase/type IV procollagenase) was not altered by IL-1 and/or Bt2cAMP. These results suggest, first, that induction of proMMP-1 and -3 synthesis may share similar transduction pathways but they are distinct from those for proMMP-2 and TIMP synthesis and, second, that cAMP does not function as a second messenger in the MMPs' induction upon IL-1 stimulation in human uterine cervical fibroblasts. Thus, it is further suggested that the system that increases the intracellular cAMP level may be involved in negative regulation of proMMP-1 and -3 production.

Interleukin 1 (IL-1) is a multifunctional cytokine that exhibits a variety of biochemical and biological activities on various cell types such as leukocytes, fibroblasts, synovial cells, chondrocytes, and hepatocytes (1–4). It mediates immune and inflammatory responses (5, 6) and acts as an important chemical mediator at inflammatory sites (4). IL-1 stimulates a variety of connective tissue cells to increase the biosynthesis of MMP-1 (vertebrate collagenase, EC 3.4.24.7); MMP-3 (stromelysin EC 3.4.24.17) (7–10); and TIMP (11–13). These enzymes and TIMP are thought to play a central role in both physiological and pathological catabolism of extracellular matrix macromolecules in the connective tissues.

Two forms of IL-1, termed α and β, were cloned, sequenced, and expressed (14-18), and recently, the cell surface IL-1 receptor was identified and characterized (19–21). Although the biochemical properties of IL-1 and its biological functions are extensively characterized, the signal transduction pathway for IL-1 is not clearly understood. Recently, it has been reported that cAMP is a second messenger of IL-1 in the induction process of interleukin 2 receptor in human YT cells (22, 23), as well as in the production of interleukin 6 in human FS-4S fibroblasts (24). Thus, the signal transduction pathway for some biological responses of IL-1 may require the cAMP pathway. On the other hand, it has been reported that IL-1 does not modify the intracellular level of cAMP in fibroblasts and chondrocytes (25), and that in pituitary cells (26) and porcine granulosa cells (27), IL-1 suppresses the forskolin- and hormone-induced cAMP accumulation. Therefore, the relationship between actions of IL-1 and the role of cellular cAMP is obscure. Clearly, such a relationship may depend upon the types of cells employed and the biological activities of IL-1 examined. Thus, we have investigated the involvement of cAMP in the signal transduction pathway of the production of IL-1-induced MMPs and TIMP, using human uterine cervical fibroblasts in culture. We have previously reported that uterine cervical fibroblasts are one of typical connective tissue cells that respond to cytokines such as IL-1 to produce much MMP-1 (10) and that the advanced collagenolysis caused by IL-1 in uterine cervix is controlled in a timely manner during cervical ripening and dilation at term pregnancy (28–30).

We report here that cAMP is not a mediator of IL-1 for induction of MMPs and TIMP, but that elevated intracellular cAMP levels suppress the IL-1-induced MMP production and slightly elevate the synthesis of TIMP in human uterine cervical fibroblasts.

EXPERIMENTAL PROCEDURES

Materials—Bt2cAMP and cAMP were purchased from Boehringer Mannheim. Theophylline was obtained from TokyoKasei Kogyo Co.,
Effects of cAMP on the IL-1-induced Matrix Metalloproteinase Synthesis

Chuo-ku Tokyo, Japan. IBMX, forskolin, alkaline phosphatase-conjugated donkey anti-(sheep IgG) IgG and rabbit anti-(mouse IgG) IgG, nitro blue tetrazolium, and 5-bromo-4-chloro-3-indolyl phosphate were products of Sigma. L-[\sup{35}S]Methionine (1,070 Ci/mmol) was purchased from American Radiolabeled Chemicals, St. Louis, MO. Protein A-Sepharose CL-4B was obtained from Pharmacia LKB Biotechnology Inc., Uppsala, Sweden. Difco, hrIL-1 (2 x 10^6 units/mg) was kindly supplied by Dainippon Pharmaceutical Co., Suita, Osaka, Japan. Monospecific sheep anti-(human synovial proMMP-1) and anti-(human synovial MMP-3) antibodies were prepared using purified corresponding enzymes (31). Mouse monoclonal anti-(bovine TIMP) IgG was purchased from Fuji Chemical Industries, Tskaksa, Toyama, Japan. Human pro-MMP-1 and pro-MMP-3 cDNAs were isolated from human rheumatoid synovial tissue (32). Gelatin was purchased from gelatin (10) and reduced and 3H-carboxymethylated transferrin (34) as respective substrates. One unit of MMP-1 or MMP-3 degrades 1 µg of MMP-1 and MMP-3 activities were measured using 12-well plates were used. After incubation, the medium was removed and the cells were washed once with methionine-free MEM without serum. After a 5-min incubation with 50% (v/v) MCM and/or BtzcAMP (1 mM), the conditioned medium (MCM) was prepared and measured using a radioimmunoassay kit (Yamasaki Co., Chiba, Japan).

Effect of hrIL-1 on Intracellular cAMP Levels in Human Uterine Cervical Fibroblasts—To examine whether IL-1 exerts its biological actions by means of cAMP, we first measured the intracellular cAMP level of human uterine cervical fibroblasts in cultures. As shown in Fig. 1, treatment of the cells with hrIL-1 (100 ng/ml) did not show any significant changes in the intracellular cAMP levels at 5 and 10 min after the treatment. On the other hand, an adenylate cyclase activator, forskolin, and a phosphodiesterase inhibitor, theophylline, increased the intracellular level of cAMP. After a 5-min incubation with forskolin (1 µM) and theophylline (1 mM), 5.7- and 1.6-fold increases over the untreated control cells were observed, respectively, and the high level of intracellular cAMP was maintained even after 10 min.

Effect of cAMP on MMP-induced MMP-1 and MMP-3 Activities—IL-1 was found to show no effect on cellular cAMP levels in human uterine cervical cells. We then examined the effect of exogenous cAMP on MMP activities. In this case, confluent fibroblasts were first treated with 50% (v/v) MCM and/or BtzcAMP (1 mM), since the augmentation of MMP

dried, and exposed to Kodak X-Omat AR x-ray film at ~80 °C.

**RESULTS**

**Effect of hrIL-1 on Intracellular cAMP Levels in Human Uterine Cervical Fibroblasts**—To examine whether IL-1 exerts its biological actions by means of cAMP, we first measured the intracellular cAMP level of human uterine cervical fibroblasts in cultures. As shown in Fig. 1, treatment of the cells with hrIL-1 (100 ng/ml) did not show any significant changes in the intracellular cAMP levels at 5 and 10 min after the treatment. On the other hand, an adenylate cyclase activator, forskolin, and a phosphodiesterase inhibitor, theophylline, increased the intracellular level of cAMP. After a 5-min incubation with forskolin (1 µM) and theophylline (1 mM), 5.7- and 1.6-fold increases over the untreated control cells were observed, respectively, and the high level of intracellular cAMP was maintained even after 10 min.

**Effect of cAMP on MCP-induced MMP-1 and MMP-3 Activities**—IL-1 was found to show no effect on cellular cAMP levels in human uterine cervical cells. We then examined the effect of exogenous cAMP on MMP activities. In this case, confluent fibroblasts were first treated with 50% (v/v) MCM and/or BtzcAMP (1 mM), since the augmentation of MMP

**Western Blotting**—Pro-MMP-1, pro-MMP-3, and TIMP in the culture media were analyzed by Western blotting. The samples were first subjected to SDS-PAGE with total 10 or 12.5% acrylamide under reducing conditions (35) and then electrotransferred onto a nitrocellulose filter. The filter was reacted with sheep anti-(human synovial proMMP-1) antibody, sheep anti-(human synovial MMP-3) antibody or mouse monoclonal anti-(bovine TIMP) antibody that was then complexed with alkaline phosphatase-conjugated donkey anti-(sheep IgG) IgG or rabbit anti-(mouse IgG) IgG. Immunoreactive pro-MMP-1, pro-MMP-3, and TIMP were visualized indirectly using 5-bromo-4-chloro-3-indolyl phosphate and nitro blue tetrazolium, as described previously (10).

**Labeling with L-[\sup{35}S]Methionine, Immunoprecipitation, and Fluorography of Pro-MMP-1 and Pro-MMP-3**—Confluent cells in 0.2% LAH/MEM were first treated with stimuli for 24 h without serum; then the medium was removed and the cells were washed once with methionine-free MEM alone and incubated with 10 µCi of L-[\sup{35}S]methionine in 1 ml of methionine-free MEM without serum. After a 2-h labeling, the medium was collected and subjected to immunoprecipitation for pro-MMP-1 and pro-MMP-3. At the same time, the cells were harvested by quick scraping with a rubber policeman and sonicated in the presence of protease inhibitors, 5 mM EDTA disodium and 200 µg/ml of proteinase inhibitors. After centrifugation at 8,000 g for 10 min, the supernatants of cell lysates were subjected to immunoprecipitation. Labeled culture media and cell extracts were incubated with 10 µl of monospecific anti-(human synovial proMMP-1 or MMP-3) antibody and then 50 µl of 15% (w/v) Protein-A Sepharose CL-4B was added. Immunoprecipitated pro-MMP-1 and pro-MMP-3 were analyzed by SDS-PAGE. Gels were impregnated with ENHANCE (Du Pont-NEN Research Products),

**Fig. 1. Accumulation of intracellular cAMP stimulated by forskolin, theophylline, and hrIL-1.** Confluent fibroblasts of the human uterine cervix at the eighth passage were incubated with forskolin (1 µM) (), theophylline (1 mM) (), hrIL-1 (100 ng/ml) (), and no treatment (C) for 5 and 10 min. After the incubation, media were removed and 7% (v/v) ice-cold trichloroacetic acid was immediately added to stop the reaction. Cellular cAMP was extracted and measured using a radioimmunoassay kit, as described under Experimental Procedures. The results are expressed as the mean of duplicate cultures.
production by MCM was more prominent than hrIL-1 when the enzymic activities of MMPs were measured (10). The apparent levels of MMP-1 and MMP-3 were measured by collagenolytic and carboxymethylated transferrin-degrading activities in the culture media, respectively (Table I). Both activities were significantly enhanced by treating the cells with MCM, but they were unchanged in the cells treated with Bt2cAMP alone. On the contrary, treating the cells with both MCM and Bt2cAMP significantly suppressed the activity of MCM-induced MMP-1 and MMP-3.

Suppression of IL-1-induced ProMMP-3 Production by cAMP and Forskolin—We then examined whether the reduced MMP activity found with MCM and Bt2cAMP is due to the decreased biosynthesis of MMP-1 and -3 molecules. The amounts of proMMP-3 synthesized and secreted under various experimental conditions were first monitored by immunoblotting using the specific antibody raised against human MMP-3. As previously reported by many investigators (11, 32, 37-39), when the cells were treated with hrIL-1, the production of proMMP-3 was enhanced 9-fold over the non-treated control cells (Fig. 2A). On the contrary, when the cells were treated with both hrIL-1 and Bt2cAMP, the Bt2cAMP suppressed the IL-1-induced proMMP-3 production in a dose-dependent manner. This suppressive effect was more prominent with theophylline.

Bt2cAMP alone did not exert any effects on the production of proMMP-3. To clarify whether their suppressive effect results from a long term action of a high level of cAMP maintained in the cells, we tested the effect of exogenous native cAMP on the IL-1-induced production of proMMP-3. As shown in Fig. 2B, cAMP (0.1 and 1 mM) reduced the IL-1-induced proMMP-3 production in a manner similar to that of Bt2cAMP treatment, although the extent was somewhat weaker as compared with Bt2cAMP. Again, cAMP alone did not modulate the basal production of proMMP-3. In addition, the accumulation of IL-1-induced proMMP-3 in the culture media was reduced almost to the basal level by co-treating the cells with a phosphodiesterase inhibitor of IBMX. To further investigate the relationship between the elevation of intracellular level of cAMP and its influence on the proMMP-3 production, the cells were treated with adenylate cyclase activator, forskolin. Forskolin alone did not show any effects on the biosynthesis of proMMP-3, but it suppressed the IL-1-induced proMMP-3 production in a dose-dependent manner. This suppressive effect was further augmented by treating the cells with both forskolin and theophylline (Fig. 2C).

Table I

| Treatment       | MMP-1 activity | MMP-3 activity |
|-----------------|----------------|----------------|
|                 | units/ml       |                |
| None            | 0.57 ± 0.14    | 0.04 ± 0.05    |
| Bt2cAMP         | 0.61 ± 0.11    | 0.24 ± 0.09    |
| MCM             | 3.270 ± 4.80a  | 1.50 ± 0.30a   |
| MCM + Bt2cAMP   | 6.49 ± 0.72b   | 0.93 ± 0.144   |

*Significantly different from nontreated control (p < 0.001).

The results suggested that both endogenous and exogenous cAMP could suppress the IL-1-induced production of proMMP-3 in

FIG. 2. Effect of Bt2cAMP, phosphodiesterase inhibitor, and adenylate cyclase activator on IL-1-induced proMMP-3 production from human uterine cervical fibroblasts. Confluent fibroblasts at the sixth passage were treated with hrIL-1 (100 ng/ml) and/or a reagent for 48 h. The harvested crude culture media were mixed with 0.2 volume of 20% (w/v) trichloroacetic acid. The precipitates were collected by centrifugation, dissolved in reducing SDS-PAGE sample buffer, and subjected to SDS-PAGE with 10% (w/v) total acrylamide. The proMMP-3 bands were visualized by immunoblotting as described under "Experimental Procedures." The amount of proMMP-3 was quantitated by densitometric scanning and calculated as a relative induction of the control. Panel A, effect of Bt2cAMP and theophylline. Lane 1, no treatment; lanes 2 and 3, Bt2cAMP (0.1 and 1 mM, respectively); lane 4, hrIL-1 (100 ng/ml); lanes 5 and 6, hrIL-1 (100 ng/ml) plus Bt2cAMP (0.1 and 1 mM, respectively); lane 7, hrIL-1 (100 ng/ml) plus theophylline (1 mM); lanes 8 and 9, hrIL-1 (100 ng/ml) plus 1 mM theophylline plus Bt2cAMP (0.1 and 1 mM, respectively). Panel B, effect of cAMP and IBMX. Lane 1, no treatment; lanes 2 and 3, cAMP (0.1 and 1 mM, respectively); lane 4, hrIL-1 (100 ng/ml); lanes 5 and 6, hrIL-1 (100 ng/ml) plus cAMP (0.1 and 1 mM, respectively); lane 7, hrIL-1 (100 ng/ml) plus 0.1 mM IBMX; lanes 8 and 9, hrIL-1 (100 ng/ml) plus 0.1 mM IBMX plus cAMP (0.1 and 1 mM, respectively). Panel C, effect of forskolin. Lane 1, no treatment; lanes 2–4, forskolin (0.01, 0.1, and 1 mM, respectively); lane 5, hrIL-1 (100 ng/ml); lanes 6–8, hrIL-1 (100 ng/ml) plus forskolin (0.01, 0.1, and 1 mM, respectively); lanes 9–11, hrIL-1 (100 ng/ml) plus 1 mM theophylline plus forskolin (0.01, 0.1, and 1 mM, respectively).
human uterine cervical fibroblasts. Similar results were observed for proMMP-1 (data not shown).

Effect of cAMP on Secretion of ProMMP-1 and ProMMP-3—To clarify whether the suppressive effect of cAMP on the IL-1-induced extracellular accumulation of proMMP-3 is due to the inhibition of zymogen secretion, we examined the possibility by biosynthetically labeling cell proteins with [35S]methionine and quantifying intracellular and extracellular levels of [35S]-labeled proMMP-1 and -3 by immunoprecipitation. As shown in the previous experiments, upon IL-1 treatment, [35S]methionine-labeled proMMP-1 and proMMP-3 were induced and accumulated in the medium (10-fold and 4.6-fold, respectively), but both Bt2cAMP and theophylline suppressed their accumulation (Fig. 3). In both cases, however, there were no significant changes in the levels of intracellular [35S]methionine-labeled proMMP-1 and proMMP-3. On the contrary, a drastic decrease in extracellular labeled enzymes and an accumulation of intracellular enzymes were observed when cells were treated with monensin to inhibit their secretion (lane 7). These results suggest that the suppression of the IL-1-induced production of proMMP-1 and proMMP-3 by Bt2cAMP and/or theophylline is not due to the inhibition of the zymogen secretion from the cells.

Suppression of IL-1-induced ProMMP-1 and ProMMP-3 mRNAs by cAMP—The effect of Bt2cAMP on the IL-1-induced accumulation of proMMP-1 and proMMP-3 mRNAs was examined by slot-blot analysis. As shown in Fig. 4, hrIL-1 significantly increased the steady-state levels of proMMP-1 and proMMP-3 mRNAs (11.6- and 12.5-fold, respectively), as compared with the control, and the IL-1-induced accumulation of proMMP-1 and proMMP-3 mRNAs was suppressed by the co-treatment of cells with hrIL-1 and Bt2cAMP, theophylline or forskolin. When cells were treated with Bt2cAMP or theophylline alone, the steady-state levels of their mRNAs did not change as compared with the control. These results indicate that the suppression of the IL-1-induced proMMPs production by Bt2cAMP or theophylline corresponded to the decrease in the steady-state levels of the respective mRNA and that cAMP regulates the IL-1-induced production of proMMP-1 and proMMP-3 at the pretranslational levels.

Stimulation of IL-1-induced TIMP Production and mRNA by cAMP—It is considered that the balance between MMPs and their specific inhibitor, TIMP, in the tissue is important for the regulation of the catabolism of extracellular matrix (40). Therefore, we also examined the effect of Bt2cAMP and theophylline on the IL-1-stimulated TIMP production. As shown in Fig. 5, hrIL-1 significantly increased the production of TIMP (1.7-fold), as reported previously (11-13). The treatment of cells with Bt2cAMP or theophylline did not suppress the TIMP production but, instead, increased it slightly: about 1.1- and 1.2-fold increases over the IL-1-treated cells were observed, respectively. The increase in TIMP production corresponded to the increase in the steady-state levels of TIMP mRNA as shown in Fig. 6. These results suggest that cAMP exerts different actions toward the IL-1-induced production of proMMP-1 and proMMP-3 and of TIMP in human uterine cervical fibroblasts.

Effect of cAMP on ProMMP-2 Production—It is known that the expression of the proMMP-2 gene is controlled differently from those of the proMMP-1 and proMMP-3 genes in many cells (41, 42). Thus, we examined the effect of cAMP on the production of proMMP-2 by zymography. As shown in Fig. 7, hrIL-1 did not show any significant alteration in the gelatinolytic activity, as compared with the control. Neither did the co-treatment of the cells with IL-1 and
were treated with hrIL-1 (100 ng/ml) and/or BhcAMP (1 mM) or theophylline (1 mM) for 24 h, and the culture media were analyzed for the production of TIMP by SDS-PAGE using 12.5% (w/v) acrylamide slab gel and immunoblotting as described under "Experimental Procedures." The bands were quantitated by densitometric scanning and calculated from nontreated control (% of control). Lane 1, no treatment; lane 2, hrIL-1; lane 3, hrIL-1 plus BhcAMP; lane 4, hrIL-1 plus theophylline; lane 5, BhcAMP; lane 6, theophylline.

**Fig. 6. Effect of BhcAMP, theophylline, and forskolin on IL-1-induced TIMP mRNA levels in human uterine cervical fibroblasts.** Confluent uterine cervical fibroblasts at the sixth passage were treated with hrIL-1 (100 ng/ml) and/or BhcAMP (1 mM), theophylline (1 mM), and forskolin (10 μM) for 24 h. Total RNA was extracted and analyzed by slot-blotting as described under "Experimental Procedures." The bands were quantitated by densitometric scanning. The relative induction of TIMP mRNA was calculated by normalizing with the amount of respective β-actin mRNA. Lane 1, no treatment; lane 2, hrIL-1 (100 ng/ml); lane 3, hrIL-1 (100 ng/ml) plus BhcAMP (1 mM); lane 4, hrIL-1 (100 ng/ml) plus theophylline (1 mM); lane 5, hrIL-1 (100 ng/ml) plus forskolin (10 μM); lane 6, BhcAMP (1 mM).

BhcAMP modulate the basal level of MMP-2 activity. This suggests that the regulation of proMMP-2 production is independent of those of proMMP-1 and proMMP-3 in human uterine cervical fibroblasts.

**DISCUSSION**

The effects of cAMP on the synthesis of MMPs have been reported by several investigators, and they were shown to be variable depending on the cell types used for the study. Kerr et al. (43) reported that cAMP inhibits the transcription of oncogene- and epidermal growth factor-induced transact (rat MMP-3) mRNA in Rat 1 cells, whereas others showed that the increase in intracellular cAMP levels induces the transcription of MMP-3 in FR3T3 cells (44) and rabbit synovial fibroblasts (45). Enhanced production of proMMP-1 by the increased level of cAMP was also reported in guinea pig macrophages (46) and in rat osteogenic sarcoma cell line UMR106-01 (47). In addition, IL-1 exerts different effects on the intracellular levels of cAMP, depending on cell type (22-27). Thus, we first examined a possible involvement of cAMP in the expression of MMP genes upon IL-1 stimulation of human uterine cervical fibroblasts. If cAMP participates in the IL-1-induced production of MMPs as a second messenger, it is anticipated that the intracellular cAMP level is altered by the IL-1 treatment. Our results, however, demonstrated that the intracellular cAMP level in the cervical fibroblasts was not affected by the hrIL-1 treatment. In addition, although high levels of intracellular cAMP were maintained by treating the fibroblasts with BhcAMP or forskolin, they did not influence the production of proMMP-1, -2, and -3. This suggests that cAMP is not involved in the inductive pathway for MMP production by IL-1. On the contrary, the treatment of the cervical fibroblasts with BhcAMP or forskolin effectively suppressed the IL-1-induced production of proMMP-1 and proMMP-3. This effect was not due to the inhibition of proMMP secretion from the IL-1-treated cells, but it was due to the decrease in the steady-state levels of proMMP-1 and proMMP-3 mRNAs. Similar suppressive effects of BhcAMP were also seen with human chorionic fibroblasts and rabbit uterine cervical fibroblasts (data not shown). These observations agree with the results of Kerr et al. (45) reporting the suppressive effects of cAMP on oncogene- and epidermal growth factor-induced transact-transcription in Rat 1 cells. In contrast to proMMP-1 and proMMP-3, the production of proMMP-2 was not affected by IL-1 and/or cAMP, and the IL-1-enhanced production of TIMP was further elevated by exogenous CAMP treatment. These observations suggest that the syntheses of proMMP-1 and proMMP-3 by connective tissue cells are regulated by a similar signaling pathway, but it is distinct from those of proMMP-2 and TIMP.

We previously reported that calmodulin acts as a suppressor of the IL-1-induced production of proMMP-1 in human uterine cervical fibroblasts (10). Such an effect of calmodulin may be related to the calmodulin-dependent increase in intracellular cAMP. The early studies by Brostrom et al. (48) demonstrated that calmodulin stimulates the adenylate cyclase activity. Since adenylate cyclase and phosphodiesterase activities are inversely regulated by the intracellular concentrations of free Ca²⁺ (49), the Ca²⁺'-calmodulin system is likely to be involved in the synthesis and degradation of cAMP. Thus, the suppressive effects of cAMP on the proMMP-1 and proMMP-3 gene expression demonstrated in this study may explain the inhibition of proMMP production by calmodulin, perhaps via a calmodulin-cAMP-dependent protein kinase system. Recently, we have also observed the suppressive effect of a specific calmodulin inhibitor, N-(6-aminophenyl)-5-
Effects of cAMP on the IL-1-induced Matrix Metalloproteinase Synthesis

chboro-1-naphthalenesulfonamide (W-7), on the IL-1-stimulated TIMP production (50). These observations indicate that the elevation of intracellular cAMP level participates in negative regulation for proMMP-1 and proMMP-3 and positive regulation for TIMP. However, the exact mechanisms of cAMP regulation for the synthesis of MMPs and TIMP are yet to be investigated.

As a part of signal transduction systems of IL-1, a rapid increase in phosphorylation of cellular proteins has been reported (51-53), but specific protein kinases responsible for the system have not been identified. Thus, the correlation between protein kinases and the production of MMPs is not fully understood. Nonetheless, our preliminary studies using several protein kinase inhibitors have suggested that protein kinase C may be closely involved in the induction process of the MMP gene expression by IL-1.2 McCaee et al. (54) have reported that protein kinase A activators such as Brt-cAMP, forskolin, and prostaglandin E1 inhibit the signal transduction system of protein kinase C by blocking the phospholipase C step in neutrotumor cell lines. In rat oligodendrocytes, it was reported that the protein kinase C-mediated phosphorylation of myelin basic protein was inhibited upon elevation of the intracellular cAMP level (55, 56). Based on these observations, it seems to be reasonable to consider that the suppressive effect of cAMP on the IL-1-induced proMMP-1 and proMMP-3 production may be reflected on the down-regulation of protein kinase C. Further studies clarifying the relationship between cAMP-calmodulin and protein kinase C are required to understand the precise actions of cAMP toward the IL-1-induced proMMP-1 and proMMP-3 production in human uterine cervical fibroblasts; these studies are now in progress.

In conclusion, we have demonstrated that cAMP is not a second messenger for IL-1 signaling in human uterine cervical fibroblasts and that it acts as a suppressor of the IL-1-induced production of proMMP-1 and proMMP-3 at pretranslational levels, but it slightly enhances the IL-1-stimulated TIMP production. cAMP did not show any effects on the production of proMMP-2. This suggests that production processes of proMMP-1 and -3, TIMP, and proMMP-2 are independently regulated. Furthermore, our results clearly indicated that intracellular mediators for the gene expression of proMMP-1 and proMMP-3 in human uterine cervical fibroblasts are different from those in FRT3 cells (44), rabbit synovial cells (45), guinea pig macrophages (46), and osteogenic sarcoma cells (47). Thus, intracellular transduction systems for the MMP gene expression appear to be variable, depending on the cell types.

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