Effects of Parathyroid Hormone (PTH) and PTH-Related Peptide on Expressions of Matrix Metalloproteinase-2, -3, and -9 in Growth Plate Chondrocyte Cultures*

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ABSTRACT

The roles of PTH and PTH-related peptide (PTH-rp) in the expression of matrix metalloproteinases (MMPs) during endochondral bone formation were investigated, using various cartilages obtained from young rabbits and rabbit chondrocyte cultures. Immunohistochemical, immunoblotting, zymographical, and/or Northern blot analyses showed that MMP-2 and -9 levels were much higher in the growth plate than in permanent cartilage in vivo. In growth plate chondrocyte cultures, PTH, PTH-rp, and (Bu)2cAMP increased the amount of MMP-2 present in the culture medium, as revealed by zymograms and immunoblots, whereas the other tested growth factors or cytokines, including bone morphogenetic protein-2 and interleukin-1, did not increase the MMP-2 level. PTH also increased the MMP-2 messenger RNA level within 24 h. In addition, PTH increased MMP-3 and -9 levels in the growth plate chondrocyte cultures. However, in articular chondrocyte cultures, PTH had little effect on the levels of MMP-2, -3, and -9. In contrast to PTH, interleukin-1 induced MMP-3 and -9, but not MMP-2, in growth plate and articular chondrocytes. These findings suggest that in ossifying cartilage, PTH/PTH-rp plays a pivotal role in the induction of various MMPs, including MMP-2 (which is considered to be a constitutive enzyme), and that PTH/PTH-rp is involved in the control of cartilage-matrix degradation during endochondral bone formation. (Endocrinology 139: 2120–2127, 1998)

IN GROWTH plates and bone fracture callus, chondrocytes undergo a sequence of changes, including proliferation, matrix synthesis, and hypertrophy. The pericellular matrix is gradually degraded as the cells become hypertrophic. Eventually the intercellular matrix is degraded and replaced by new bone. This programed matrix degradation is essential for elongation and repair of the skeleton. In growth plates, the rate of cartilage matrix degradation is equal to the rates of proliferation, matrix formation, and hypertrophy. This coordination is required for the maintenance of the growth plate width and continuous bone elongation until puberty.

Many hormones and growth factors have been shown to modulate the proliferation, matrix synthesis, and expression of the hypertrophy-related phenotypes (alkaline phosphatase, 1,25-dihydroxyvitamin D3 receptor, and type X collagen) (1–12). The hormones and growth factors that regulate cartilage matrix degradation before ossification, in contrast, are unknown.

Matrix metalloproteinase (MMP) is thought to be crucial for cartilage matrix degradation, because MMP inhibitors prevent cartilage resorption and proteoglycan loss in arthritic joints in vivo (13, 14). MMP-1 (collagenase) degrades native collagen; MMP-2 (gelatinase A) and -9 (gelatinase B) degrade gelatin, elastin, and fibronectin; and MMP-3 (stromelysin-1) degrades proteoglycan, link protein, laminin, and fibronectin at neutral pH (15).

We are interested in the actions of PTH and PTH-related peptide (PTH-rp) on MMP, because PTH/PTH-rp plays an important role in endochondral bone formation. PTH/PTH-rp enhances DNA and aggrecan synthesis in growth plate chondrocytes (3, 16, 17) and suppresses their hypertrophy and apoptosis in vitro and in vivo (9, 11, 17–23). PTH-rp is synthesized in various tissues, including cartilage and perichondrium (17, 20), and binds to the PTH/PTH-rp receptor (24). The PTH/PTH-rp receptor level is much higher in the growth plate than in permanent cartilage (9, 25). Furthermore, a null mutation of the PTH-rp gene causes dwarfism and growth plate dysplasia without appreciable histological changes in articular cartilage and nonskeletal tissues (11, 17). The crucial role of PTH/PTH-rp in endochondral bone formation is supported by our observations. In a previous study, we showed that PTH and PTH-rp increased the amount of MMP-2 in the growth plates of young rabbits (26). In this study, we examined the role of PTH and PTH-rp in the expression of MMP-2, -3, and -9 in growth plate chondrocyte cultures.

Received October 27, 1997.
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* This work was supported in part by funds from Chugai Pharmaceutical Co., Sumitomo Pharmaceutical Co., the Ciba-Geigy Foundation (Japan), and the Growth-Science Foundation (Japan).
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bone formation is also indicated by a link between a constitutively active mutation of the PTH/PTHrP receptor gene and Jansen-type metaphyseal chondrodysplasia (26).

We report here that PTH/PTHrP induces MMP-2, -3, and -9 in growth plate chondrocyte cultures, whereas it has little effect on MMP levels in articular chondrocyte cultures. The enhancement of these major MMPs by PTH/PTHrP may be involved in the programmed cartilage matrix degradation during endochondral bone formation.

Materials and Methods

Materials

Human recombinant PTH-(1–84), PTH-(1–84), and PTH-(1–141); human PTH-(15–34); and 1,25-dihydroxyvitamin D₃ were supplied by Dr. K. Sato (Chugai Pharmaceutical Co., Tokyo, Japan). Human transforming growth factor-β1 (TGFβ1) was obtained from R&D Systems (Minneapolis, MN). Human insulin-like growth factor I (IGF-I; recombinant), insulin, and epidermal growth factor (EGF) were purchased from Wako Pure Chemical Industry (Osaka, Japan). Human basic fibroblast growth factor (bFGF) was purchased from Cosmo Bio (Tokyo, Japan). Human recombinant interleukin-1β (IL-1β) was supplied by Dr. Y. Hirai (Ohtsuka Pharmaceutical Co., Tokushima, Japan). Human bone morphogenetic protein-2 (BMP-2; recombinant) was a gift from Dr. J. M. Wozney (Genetics Institute, Cambridge, MA) and Dr. K. Takahashi (Yamanouchi Pharmaceutical Co., Tokyo, Japan). T₃ and (Bu)₂cAMP were purchased from Sigma Chemical Co. (St. Louis, MO). Eagle’s medium, α-modification (αMEM), and FBS were obtained from Sanko Jyueyaku (Tokyo, Japan) and Mitsubishi Kagaku (Tokyo, Japan), respectively.

Immunohistochemistry studies

Paraffin sections (4 μm thick) of rib cartilage obtained from 4-week-old male Japanese White rabbits were incubated at room temperature with 1 U/ml chondroitinase from avian-biotin-peroxidase complex (Seikagaku Kogyo, Tokyo, Japan) in 50 mM Tris-HCl, pH 7.4, containing 0.15 M NaCl for 3 h. These sections were incubated with a mouse monoclonal antibody (mAb; IgG) to human MMP-2 (42-5D11), MMP-9 (56-2A4), or type II collagen (II-4C11; 10 μg/ml) overnight at 4°C. The sections were washed with PBS, incubated for 1 h after blocking of endogenous peroxidase with 0.3% H₂O₂ and 0.1% NaN₃ in methanol and blocking of nonspecific IgG binding with 5% normal horse serum. The sections were washed with PBS, incubated with a biotinylated horse IgG to mouse IgG (diluted 1:200; Vector Laboratories, Burlingame, CA) for 30 min, then stained with an avidin-biotin-peroxidase complex (Vector Laboratories). Color was developed with 0.03% 3,3′-diaminobenzidine tetrahydrochloride in 50 mM Tris-HCl, pH 7.6, containing 0.006% H₂O₂, and the sections were counterstained with hematoxylin.

Chondrocyte cultures

Chondrocytes were isolated from the rib growth plate and the surface (0.2 mm) of articular cartilage of the femur at knee joints of 4-week-old male Japanese White rabbits, as previously described (27, 28). The cells were seeded at 2 × 10⁶, 3 × 10⁶, or 10⁷ cells/35-, 16-, or 6-mm plastic culture dishes, respectively, and maintained in 2, 0.5, or 0.1 ml αMEM supplemented with 10% FBS, 50 μg/ml ascorbic acid, 32 U/ml penicillin, and 40 μg/ml streptomycin (medium A) at 37°C under 5% CO₂ in air. The cultures were fed fresh medium A 3 days after seeding, and thereafter the medium was changed every other day.

Determination of alkaline phosphatase activity

Chondrocytes grown in 16-mm wells were homogenized in a glass homogenizer in 1 ml 0.9% NaCl-0.2% Triton X-100 at 4°C and centrifuged for 15 min at 12,000 × g. Alkaline phosphatase activity in the supernatant was measured by a modification of the method of Bessey et al., using p-nitrophenyl phosphate as the substrate, as described previously (2, 29). One unit was defined as the activity catalyzing hydrolysis of 1 μmol p-nitrophenol phosphate/μg DNA/30 min.

Zymography

Chondrocytes in 16-mm wells were exposed to 0.5 ml serum-free αMEM supplemented with various hormones, growth factors, or cytokines for 48 h. The media (1 or 4 μg protein/lane) conditioned by chondrocytes were mixed with concentrated Laemmli buffer (30) without reducing agent. Protein samples were resolved in 10% polyacrylamide gels containing 0.5 mg/ml gelatin (Wako Pure Chemical Industry, Osaka, Japan) or 0.5 mg/ml casein (Wako Pure Chemical Industry) by SDS-PAGE. After the gels were washed in 2.5% Triton X-100 for 30 min to remove SDS and in 50 mM Tris-HCl (pH 8.0) for 10 min, they were incubated for 8–24 h in 50 mM Tris-HCl (pH 8.0) containing 5 mM CaCl₂, 0.2 mM NaCl, and 0.02% NaN₃ at 37°C. The gels were then stained with Coomasie brilliant blue. The enzymatic activity was seen as negatively stained bands.

Sequential slices (the width of a slice, ~0.3 mm; slice 1, the hypertrophic zone; slice 2, the matrix-forming zone; slice 3, the proliferating zone) of the rib growth plate were obtained from three 4-week-old male Japanese White rabbits, as described previously (9, 28). Resting cartilage of the rib and articular cartilage of the femur at knee joints was also obtained from three 4-week-old male Japanese White rabbits (9, 28). The tissue was minced and homogenized in 10 vol Laemmli buffer (30). Protein in the samples (2 μg protein/lane) was resolved in the gels containing gelatin by SDS-PAGE, as described above.

Immunoblotting

Chondrocytes in 6-mm wells were exposed to 0.1 ml serum-free αMEM supplemented with various hormones, growth factors, or cytokines for 48 h. The media conditioned by chondrocytes were mixed with concentrated Laemmli buffer (30) without reducing agent.

The sequential growth plate slice and resting cartilage of the rib and articular cartilage of the femur at knee joints obtained from three 4-week-old male Japanese White rabbits were minced and homogenized in 10 vol sodium acetate buffer, pH 5.8, containing 4 μg guanidine HCl, 1 mM phenylmethylsulfonylfluoride, 10 μM amidoprophylsulfonylfluoride, 10 mM N-ethylmaleimide, 1 mM EDTA, and 0.1 mM pepstatin A in a Polytron homogenizer (Kinematica, Littau, Switzerland) at 4°C. The homogenate was incubated at 4°C for 18 h, then centrifuged at 5,000 × g for 10 min at 4°C. The supernatant was dialyzed against a solution containing protease inhibitors (10 μM amidoprophylsulfonylfluoride, 10 mM N-ethylmaleimide, 10 μM pepstatin A, and 1 mM EDTA) at 4°C, freeze-dried, and then mixed with Laemmli buffer (30) without reducing agent.

Proteins (2 μg/lane) in the samples (the conditioned media or cartilage extracts) were resolved in a 4–20% polyacrylamide gradient gel by SDS-PAGE under nonreducing conditions, then electrophoretically transferred to a nitrocellulose membrane (31). Nonspecific binding was blocked in a milk solution (5% nonfat dry milk in PBS) at room temperature for 1 h. The blots were exposed to a primary mouse mAb against human MMP-2 (42-5D11, Fuji Chemical Industries) at 4°C for 12 h (32) and then exposed to 125I-labeled sheep antimouse IgG-F(ab')₂ fragment (Amersham, Aylesbury, UK) in PBS for 3 h at room temperature. (Antibodies that cross-react with rabbit MMP-3 or -9 in immunoblots are not available.)

Northern analysis

Total RNA was extracted by the guanidine HCl method (33). Samples of total RNA (5–15 μg) were denatured in the presence of 2.2 M formaldehyde, electrophoresed on a 1% agarose gel containing formaldehyde, and transferred to a Nitran membrane filter (Schleicher and Schuell, Keene, NH) (34). The RNA bound to the Nitran filter was prehybridized at 68°C with buffer containing 6 × SSC, 0.5% SDS, 5 × Denhardt’s solution, 0.1 mg/ml sonicated salmon DNA, and 10 mM EDTA for 1 h. The hybridization was carried out at 68°C for 15 h in the same buffer with a 32P-labeled 1.5-kilobase (kb) human MMP-2 complementary DNA (cDNA) probe (35) or a 32P-labeled 0.6-kb rabbit glyceraldehyde-3-phosphate dehydrogenase (GAPDH) cDNA probe, which was labeled with an oligolabeling kit (Pharmacia, Japan, Tokyo). The

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membrane was washed with 0.2 × SSC containing 0.1% SDS and exposed to x-ray film.

**RT-PCR/Southern blot analysis**

MMP-3 cDNA (336 bp) and GAPDH cDNA (613 bp) were obtained by RT-PCR from total RNA of rabbit cartilage using pairs of oligonucleotides: 5′-CTGGAGGTTCATGAGAAGA-3′ and 5′-CAGTCTCAT-GCTGGAGATTCC-3′ for MMP-3, and 5′-GTCACGGTGAAGAAGG-3′ and 5′-GCTTTACCCACCTCTCTTGAT-3′ for GAPDH. These were synthesized based on the nucleotide sequences of rabbit MMP-3 (36) and rabbit and human GAPDH cDNAs (37), respectively. The PCR products were subcloned into pCRII vector (Stratagene, La Jolla, CA) and identified by sequencing by means of dideoxy chain termination (38).

The MMP-3 and GAPDH messenger RNA (mRNA) levels were estimated by RT-PCR/Southern blotting using the pairs of primers. The PCR products (25 and 28 cycles for MMP-3 and GAPDH, respectively) were separated on a 1% agarose gel, then transferred to Nytran. The MMP-3 and GAPDH cDNAs were labeled with an oligolabeling kit (Pharmacia Japan, Tokyo, Japan). Hybridization proceeded under the same conditions as those described above.

**Gelatinolytic and stromelysin activities in conditioned media**

Chondrocytes in 16-mm wells were incubated with 0.5 ml αMEM supplemented with various concentrations of hormones or cytokines for 48 h. The assay for gelatinase was carried out using conditioned media in the presence of 1 mM 4-aminophenylmercuric acetate (an activator of latent MMPs) against heat-denatured 14C-acetylated collagen (type I) as a substrate, as previously described (39). For the determination of stromelysin activity, the media were preincubated with 1.5 mM 4-aminophenylmercuric acetate at 37 °C. The stromelysin activity was then measured using reduced and carboxymethylated [3H]transferrin as a substrate, as previously described (40). MMP-3, but not the other MMPs tested, degraded transferrin (40). These assays were performed in the presence of 2 mM phenylmethylsulfonylfluoride and 5 mM N-ethylmaleimide to inhibit serine and cystein proteinases. One unit of these enzymes degrades 1 μg substrate/min at 37 °C.

**Results**

**Localization of MMP-2 and -9 in the growth plate**

As the rate of matrix degradation is much higher in the growth plate than in the resting cartilage or articular cartilage, we compared MMP levels in these cartilages. Our immunohistochemical studies detected MMP-2 in the proliferating, matrix-forming, and hypertrophic zones of the growth plate and MMP-9 in the hypertrophic zone. MMP-2 or -9 was not detected in the resting zone (Fig. 1, a and b). The growth plate and resting cartilage equally reacted with antitype II collagen mAb (data not shown). No staining was evident in the cartilage samples incubated with control IgG (Fig. 1c).

We next examined the distribution of MMP-2 and -9 in various cartilage regions by biochemical means (Fig. 2). The protein in the hypertrophic zone (slice 1), the matrix-forming zone (slice 2), and the proliferating zone (slice 3) of the growth plate as well as the resting zone and articular cartilage was extracted with 4 M guanidine or Laemmli’s buffer. Immunoblotting analyses of the guanidine extracts showed that although the level of pro-MMP-2 (66 kDa) was almost constant among the growth plate slices, it was much higher in the growth plate than in the resting zone or articular cartilage (Fig. 2a). The zymographical analyses of the SDS extracts also showed that the level of pro-MMP-2 was much higher in the growth plate than in the resting zone or articular cartilage.
cartilage (Fig. 2b). In addition, Fig. 2b shows that pro-MMP-9 (92 kDa) was expressed in the hypertrophic zone. Rabbit MMP-2 has two species of mRNA (about 3.7 and 2.9 kb) that are expressed constitutively in several tissues (41). Northern analyses showed that the level of the MMP-2 transcripts (3.7 and 2.9 kb) was much higher in the growth plate than that in the resting zone or articular cartilage (Fig. 2c).

**Effects of PTH on MMP-2 expression by chondrocytes**

The zymographical and immunoblotting analyses showed that in triplicate cultures of rabbit growth plate chondrocytes, PTH increased the secreted levels of 66-kDa pro-MMP-2 and 62-kDa MMP-2 (an active form that is produced by partial proteolysis of pro-MMP-2) in the absence of serum (Fig. 3, a and b). The zymographical analyses did not provide quantitative data, but detected both 66- and 62-kDa MMP-2. On the other hand, the immunoblotting analyses did not detect 62-kDa MMP-2 at low levels. The lack of the 62-kDa band in some samples seems to be due to a low sensitivity of the antibody (Fig. 3, b and c).

The zymographical analysis showed that scarcely any gelatinolytic activity was detected in the cell matrix layers, indicating that the majority of the MMP was secreted into the medium (data not shown).

Figure 3c shows that the effect of PTH on MMP-2 levels could be observed at 10^{-8} M and reached a maximum at 10^{-8} M.

Figure 4a shows that PTH increased the level of MMP-2 transcripts in the growth plate chondrocytes at 24 and 48 h. As cAMP is partly involved in mediating the action of PTH on chondrocytes (3, 42), (Bu)_2cAMP (a membrane-permeable analog of cAMP) increased the level of MMP-2 transcripts at 24 and 48 h (Fig. 4b). (Bu)_2cAMP also increased the protein level of pro-MMP-2 (Fig. 5). However, TGFβ, BMP-2, bFGF, EGF, T3, 1,25-dihydroxyvitamin D3, IL-1, IGF-I, and insulin did not increase the pro-MMP-2 level (Fig. 5).

**Effects of PTH on the expression of MMP-9**

As MMP-9 was expressed in the hypertrophic zone in vivo (Figs. 1b and 2b), we examined the effect of PTH on MMP-9 expression by cultured chondrocytes on days 14 (the matrix-forming and early hypertrophic stage) and 28 (the hypertrophic stage). We used chondrocytes derived from the entire rib growth plate. Thus, the initial cell population is heterogeneous. Nonetheless, these cells reinitiate proliferation and are capable of recapitulating all stages of differentiation in vitro (43). Previous studies have shown that rabbit chondrocytes from the entire rib growth plate undergo proliferation, matrix formation, and hypertrophy in vitro, and that the cell changes proceed in the same order as those in the growth plate in vivo (44). In these cultures, alkaline phosphatase activity increases from day 12 and reaches a maximum on day 28 (44). In the present study, the cultures became confluent on day 7, and the alkaline phosphatase activity on day 28 (2.6 U) was 3-fold higher than that (0.9 U) on day 14. PTH induced 92-kDa MMP-9 in the chondrocyte cultures on day 28 in all independent studies (Fig. 6; data not shown), but this effect of PTH on MMP-9 was not always observed on day 14 (Fig. 3a; data not shown). On the other hand, PTH induced MMP-2 expression on days 14 and 28 in all independent studies (Figs. 3a and 6; data not shown). In another series of studies, PTH induced MMP-2, but not MMP-9, on day 10 before the induction of alkaline phosphatase (data not shown). These findings suggest that PTH enhances MMP-2 synthesis throughout the matrix-forming and hypertrophic stages and stimulates MMP-9 synthesis after chondrocytes becoming hypertrophic.

On day 28, IL-1β induced MMP-9 in growth plate chondrocytes, as expected from previous studies (45), although it had little effect on the MMP-2 level (Fig. 6). In this experiment
MMP-2 mRNA expression in chondrocyte cultures. Growth plate chondrocytes were incubated in serum-free αMEM supplemented with (Bu)2cAMP (1 mM; cAMP), TGFβ (10 ng/ml), BMP-2 (50 ng/ml; BMP), bFGF (10 ng/ml; FGF), EGF (30 ng/ml), Tb (10−8 M), 2,5-dihydroxyvitamin D3 (10−8 M; D3), IL-1 (10 ng/ml), IGF-I (50 ng/ml; IGF), or insulin (10 μg/ml; Ins) on day 14 and then incubated for 48 h. Proteins in the media (1 μg/lane) were subjected to immunoblotting analysis.

Effects of PTH on the expression of MMP-3

As MMP-3 does not effectively degrade gelatin, this enzyme activity was measured by casein zymography. The casein zymograms showed that the addition of PTH induced stromelysin activity at a position corresponding to 55 kDa (55-kDa MMP, probably pro-MMP-3) dose dependently within 48 h (Fig. 8a). In this assay, PTH also increased the MMP-2 level dose dependently (Fig. 8a). Furthermore, PTH induced the expression of MMP-3 mRNA in chondrocytes within 24 h (Fig. 8b).

Comparison between effects of PTH and IL-1 on production of gelatinase-like (MMP-2 plus -9) and stromelysin-like (MMP-3) activities by chondrocytes

On days 7 (the proliferative stage), 14 (the matrix-forming and early hypertrophic stage), and 28 (the hypertrophic stage), growth plate chondrocytes were incubated for 48 h in the absence (Fig. 9a, open bars) or presence of PTH (closed bars) or IL-1β (hatched bars). The gelatinase activity (upper panel) and stromelysin activity (lower panel) released into the media were determined, using 14C-acetylated gelatin or reduced
and carboxymethylated [3H]transferrin as a substrate, respectively (39, 40).

In the absence of PTH and IL-1, gelatinase activity was undetectable on day 7, although it was present at low levels on days 14 and 28 (Fig. 9a, upper panel). In the absence of PTH and IL-1, stromelysin activity was undetectable on days 7, 14, and 28 (lower panel). The addition of PTH markedly enhanced the production of gelatinase activity on days 14 and 28 (upper panel). PTH also enhanced the production of stromelysin activity on day 28 (lower panel). The effect of PTH on gelatinase production was similar to that of IL-1β (upper panel), whereas PTH was less effective on stromelysin production than was IL-1β (lower panel).

In cultures of articular chondrocytes from the same rabbits, PTH had little effect on the production of gelatinase (Fig. 9a, upper panel) or stromelysin (Fig. 9a, lower panel), although IL-1β induced the production of both gelatinase and stromelysin by the cells. The gelatin zymograms showed that PTH had little effect on the level of MMP-2 or -9 in articular chondrocytes, whereas IL-1β induced MMP-9 in the cultures (Fig. 9b). These findings differentiated the action of PTH on MMPs from that of IL-1.

**Discussion**

In this study, we found that MMP-2 and -9 levels are much higher in the growth plate than in permanent cartilage. Previous studies have shown that MMP-1 and -3 levels and collagenase activity increase during the hypertrophic stage in vitro and in vivo (46, 47). The factors that up-regulate MMP synthesis during endochondral bone formation are not known. However, PTH induced MMP-2, -3, and -9 in rabbit growth plate chondrocyte cultures. PTH also induced collagenase production in some, but not all, cultures of growth plate chondrocytes (Satakeda, H., and Y. Kato, unpublished data). These findings suggest that PTH/PTH-rp is involved in the induction of various MMPs in the growth plate.

Unlike other MMPs, MMP-2 is constitutively expressed in several tissues and is not usually induced by inflammatory stimuli (41, 48). In almost all evaluated cells, many growth factors and cytokines do not enhance MMP-2 synthesis even when they induce other MMPs in vitro (48–51). Thus, MMP-2 may be involved in normal turnover of the extracellular matrix in several tissues. However, in a few cell types, TGFβ and IL-1 enhance MMP-2 synthesis. TGFβ stimulates MMP-2 synthesis in gingival fibroblasts and some tumor cells (50, 51), and IL-1 stimulates MMP-2 synthesis in glomerular mesangial cells (49). PTH induced MMP-2 in growth plate chondrocytes. These findings suggest that MMP-2 is involved in the remodeling of several tissues in some situations.

The action of PTH/PTH-rp on MMP may be critical, particularly for the control of cartilage matrix degradation during endochondral bone formation, because PTH/PTH-rp had no effect on the level of MMP-2, -3, or -9 in cultures of
Fig. 9. Effects of PTH and IL-1 on gelatinase and stromelysin activities in cultures of growth plate chondrocytes and articular chondrocytes. a, On days 7, 14, and 28, chondrocytes in 16-mm wells were transferred into 0.5 ml serum-free MEM and incubated for 48 h in the absence (open bars) or presence of 10^{-8} M PTH (closed bars) or 3 ng/ml IL-1β (hatched bars). The enzyme activities in the medium were measured after treatment with 4-aminophenylmercuric acetate. Values are the average ± SD of four cultures. Similar results were obtained in three independent studies (not shown). b, On day 28, articular chondrocytes were not exposed or were exposed to 10^{-8} M PTH or 3 ng/ml IL-1β in the absence of serum and incubated for 48 h. MMP in the media was analyzed by gelatin zymography.

articular chondrocytes that did not undergo endochondral bone formation. The selective action of PTH/PTH-rp on growth plate chondrocytes is partly explained by the 10-fold increases in PTH/PTH-rp receptor (41,000 receptors/cell) (9) and its mRNA (25) levels in the growth plate relative to those in permanent cartilage.

If the PTH-rp stimulation of MMP synthesis observed in vitro is relevant to matrix degradation in vivo, then PTH-rp-depleted mice should show an abnormal accumulation of the matrix in the growth plate. This is indeed what occurs. In the PTH-rp-depleted growth plate, type II collagen is abnormally accumulated in the hypertrophic zone. A fraction (~40%) of the terminal chondrocytes (alkaline phosphatase-producing cells) are unable to increase their cell size, perhaps because of impaired degradation of the pericellular matrix. The small terminal cells, but not the large ones, are surrounded by an intact type II collagen matrix (17). In addition, the chondrocyte lacunae in the PTH-rp-depleted growth plate become more resistant to vascular invasion (17). These findings taken together with our present observations suggest that PTH-rp promotes chondrocyte-mediated matrix degradation during endochondral bone formation in vivo. IL-1 induces MMP-1, 3, and -9 in various chondrocytes (45, 52, 53), and this action of IL-1 is thought to be crucial for cartilage matrix degradation in arthritic cartilage. However, in contrast to PTH, IL-1 inhibits the proliferation (54) and differentiation (19, 53) of chondrocytes at all stages and has little effect on MMP-2 synthesis by growth plate chondrocytes. Thus, IL-1 may not be crucial for the cartilage resorption before ossification.

It is noteworthy that unlike PTH/PTH-rp, bFGF and 1,25-dihydroxyvitamin D₃ had little effect on the MMP-2 level in growth plate chondrocyte cultures, although these compounds are as potent as PTH/PTH-rp in inhibiting chondrocyte hypertrophy in vitro and in vivo (6, 12, 28, 55). These findings suggest that PTH/PTH-rp and the other inhibitors of hypertrophy have different roles in the remodeling of the extracellular matrix during endochondral bone formation.

In growth plates, PTH/PTH-rp enhances DNA synthesis at the proliferative stage (16, 17), stimulates the syntheses of aggrecan (3) and type II collagen at the matrix-forming stage (56), and induces MMP-2, -3, and -9 at the hypertrophic stage. PTH-rp stimulation of the synthesis and degradation (turnover) of the cartilage matrix in the growth plate may facilitate bone elongation.

In conclusion, the findings in the present study showed that PTH/PTH-rp markedly enhances the expressions of MMP-2, -3, and -9 in maturing chondrocytes. This action of PTH/PTH-rp may be critical in the control of programmed cartilage resorption during skeletal development and repair.

Acknowledgment

We thank the Research Center for Molecular Medicine, Hiroshima University School of Medicine, for the use of their facilities.

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