Potent Mitogenic Effects of Parathyroid Hormone (PTH) on Embryonic Chick and Rabbit Chondrocytes

Differential Effects of Age on Growth, Proteoglycan, and Cyclic AMP Responses of Chondrocytes to PTH

Tatsuya Koike, Masahiro Iwamoto, Atsushi Shimazu, Kazuhisa Nakashima, Fujio Suzuki, and Yukio Kato
Department of Biochemistry, Faculty of Dentistry, Osaka University, 1-8, Yamadaoka, Suita, Osaka 565, Japan

Abstract

The effect of PTH on chondrocyte proliferation as a function of cartilage age was examined. PTH[1–34] induced a 12- to 15-fold increase in the efficiency of colony formation in soft agar by chondrocytes from embryonic 13- to 19-d-old chickens and fetal 25-d-old rabbits with a 10-fold increase in their DNA content. It also caused a 2.5-fold increase in [^{3}H]thymidine incorporation into DNA in fetal 25-d-old rabbit chondrocytes. No mitogenic responses to PTH were observed, however, in postnatal 7- to 21-d-old chick chondrocytes or postnatal 21-d-old rabbit chondrocytes. This age dependency was observed only with PTH: fibroblast growth factor, epidermal growth factor, and insulin stimulated chondrocyte proliferation irrespective of cartilage age. The absence of a mitogenic effect in postnatal chondrocytes was not due to a decrease in number or a reduction in affinity of receptors for PTH. PTH also increased [^{35}S]sulfate incorporation into proteoglycans and the cyclic AMP level in fetal and postnatal chondrocytes, but at 100-fold higher concentrations (10^{-8}–10^{-7} M) than those (10^{-10}–10^{-9} M) required for the stimulation of cell division. These results suggest that PTH is a potent mitogen for embryonic chondrocytes, and that its mitogenic effect disappears selectively after birth. (*J. Clin. Invest.* 1990. 85:626–631.)
chondrocyte • PTH • growth

Introduction

PTH is one of the most important hormones for regulation of the blood calcium level (for review, see reference 1). In addition, PTH has direct anabolic effects on chondrocytes. Suzuki et al. have shown that PTH causes a 1.5-fold increase in [^{35}S]-sulfate incorporation into glycosaminoglycans in 6-wk-old rat costal chondrocytes in monolayer cultures (2). This increase in [^{35}S]sulfate incorporation was due to enhanced synthesis of large, chondroitin sulfate proteoglycans, the main component of cartilage matrix (3, 4). PTH also increased the cAMP level 50-fold (4–6) and the ornithine decarboxylase activity 5-fold (5, 7) in 4- to 7-wk-old rabbit costal chondrocytes in monolayer cultures. However, it did not enhance [^{3}H]thymidine incorporation into DNA in postnatal chondrocytes (5). On the other hand, there are reports that PTH caused a 1.5- to 2-fold increase in the length, weight, and DNA content of embryonic 9-d-old chicken femur and pelvic cartilage in organ culture (8, 9). A 1.5-fold increase by PTH in [^{3}H]thymidine uptake was also noted in embryonic cartilage (9). These conflicting observations about the mitogenic action of PTH can be explained by supposing that the mitogenic action of PTH is dependent on cartilage age.

In this work, we tested this supposition by comparing the growth responses of embryonic chicken sternal and rabbit costal chondrocytes to PTH with those of postnatal chondrocytes. In these experiments PTH increased the efficiency of colony formation by embryonic rabbit and chick chondrocytes in soft agar 12- to 15-fold, but did not stimulate that of postnatal 7- to 21-d-old rabbit and chick chondrocytes. PTH also increased [^{3}H]thymidine incorporation into DNA in fetal rabbit chondrocytes in monolayer cultures, but decreased its incorporation into postnatal chondrocytes. Thus, the difference observed in previous studies with chondrocytes in monolayer and organ cultures proved to be functions of the age of cartilage cells rather than the species, or in vivo location of the cartilage, or the culture conditions. The present study also showed that PTH stimulated the syntheses of proteoglycan and cAMP in both fetal and postnatal chondrocytes. These results suggest that the growth response of chondrocytes to PTH, but not their differentiation response, is developmentally regulated.

Methods

Materials. A bovine PTH-active fragment (synthetic 1–34, PTH[1–34]) and [Nle^8, Nle^{18}, Tyr^{34}]bovine PTH[1–34] were purchased from Peninsula Laboratories, Inc. (Belmont, CA). Basic fibroblast growth factor (bFGF; from bovine pituitary)[1] was generously supplied by Dr. Denis Gospodarowicz, University of California, San Francisco. Epidermal growth factor (EGF) was from Toyobo Co., (Osaka, Japan); insulin and collagenase (type IA) were from Sigma Chemical Co. (St. Louis, MO); Bacto agar and tryptose phosphate broth were from Difco laboratories (Detroit, MI); fetal bovine serum was from Gibco Laboratories (Grand Island, NY); Ham's F-12 medium and Eagle's MEM were from Nissui Pharmaceutical Co. (Tokyo, Japan). Plastic culture dishes were obtained from Terumo Co. (Tokyo, Japan). [^{35}S]Sulfate (carrier-free) was purchased from Japan Atomic Energy Research Institute (Tokyo, Japan) and [6-^{3}H]thymidine (5 Ci/mmol) from Amersham International (Amersham, UK). A cAMP assay kit was purchased from Yamasa Shoyu Co. (Chiba, Japan).

Chondrocyte. Rabbit chondrocytes were isolated from growth plates of ribs of fetal 25-d-old (5–6 d before birth) and 3- to 4-wk-old

Address correspondence to Dr. Yukio Kato, Osaka University, Faculty of Dentistry, Department of Biochemistry, 1-8, Yamadaoka, Suita, Osaka 565, Japan.

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1. *Abbreviations used in this paper:* bFGF, basic fibroblast growth factor; EGF, epidermal growth factor; IGF-1, insulin-like growth factor 1; ^{125}I-PTH[1–34], ^{125}I-[Nle^8, Nle^{18}, Tyr^{34}]bovine PTH[1–34]; TGF-beta, transforming growth factor-beta.

postnatal male New Zealand rabbits. Fetal and postnatal cartilage segments were incubated for 2.5 h with 1 and 2 mg/ml of collagenase (Sigma type IA), respectively, as described previously (10, 11).

Chicken chondrocytes were obtained from embryonic 13- to 19-d-old or postnatal 3- to 14-d-old chicken sternal cartilages (12). Chicken cartilages were incubated at 37°C for 1 h in 10 ml of PBS (calcium- and magnesium-free) containing 2.5 mg/ml of trypsin. After incubation they were cut into small pieces. The tissue fragments were incubated in 10 ml of PBS containing 1 mg/ml of collagenase and 2.5 mg/ml of trypsin at 37°C. After 1.5 h single cell suspensions were obtained by pipetting the cell aggregates up and down in a 10-ml pipette. Cells were seeded at a density of 10^6 cells/10 cm diameter in uncoated plastic dishes, and maintained in 10 ml of Ham's F-12 medium supplemented with 10% fetal bovine serum, 40 μg/ml of streptomycin, and 32 U/ml of penicillin under 5% CO_2 in air at 37°C for 6 d before experiments. The purity of the chondrocyte population was > 99%. No syntheses of nonchondrogenic collagens such as type I could be detected in similar chondrocyte cultures (13, 14).

Colony formation in soft agar. A basal layer of 0.5 ml of 0.72% Bacto agar in Ham's F-12 medium supplemented with 10% fetal bovine serum, 0.3% tryptose phosphate broth, and 60 μg/ml kanamycin (referred to as medium A) was introduced into 16-mm plastic petri microwells. Chondrocytes (5,000 cells) were suspended in 0.5 ml of 0.41% agar in medium A and overlaid on the basal layer. Hormones and growth factors were dissolved in PBS (1–5 μl) with 0.1% BSA, and added every 4 d beginning 3 d after cell seeding. Addition of PBS (1–5 μl) containing 0.1% BSA alone had no effect. On days 7, 14, and 21, three types of colonies were counted separately: compact, small colonies (0.1–0.2-mm-diam); closely packed, large colonies (> 0.2 mm); and loose colonies composed of > 40 cells. Colonies were optically counted with a 2-mm grid in a glass plate using a phase-contrast microscope. Counts in most experiments were made separately by two investigators.

DNA content of soft agar cultures. The DNA content of cultures in soft agar was determined by the method of Benya and Shaffer (15). Whole cultures were treated with pronase E (protease, 2 mg/ml, 24 h, at 55°C; Sigma Chemical Co.), incubated with 0.1 M NaOH and 0.2% Triton X-100 to extract the DNA, cooled at 4°C for 24 h, and centrifuged (50,000 rpm, 30 min, Beckman 50Ti rotor; Beckman Instruments, Inc., Palo Alto, CA) to remove the agar and residue. 0.5-ml aliquots of the pronase supernatant were brought to a final concentration of 12.5% TCA and stood in ice for 2 h. The preparations were then centrifuged at 4°C in a microfuge and the precipitates were washed once with cold 10% TCA and twice with ethanol/diethyl ether (3:1 vol/vol). The residues were solubilized in 0.1 N NaOH containing 0.2% Triton X-100 and their DNA contents were assayed by a fluorometric method (16). Background fluorescence was measured using soft agar cultures without cells.

Monolayer cultures. For preparation of low-density cultures on plastic tissue culture dishes, rabbit chondrocytes were seeded at 4×10^4 cells per 16-mm-diam plastic well, and maintained in 0.5 ml MEM supplemented with 60 μg/ml of kanamycin and 10% fetal bovine serum under 5% CO_2 in air at 37°C.

DNA synthesis. When cultures maintained on 16-mm wells became confluent, the cells were preincubated for 24 h in 0.5 ml of MEM with 0.5% fetal bovine serum. The cells were then transferred to 0.5 ml of the same medium supplemented with PTH[1–34] or growth factors. After 24 h [^{3}H]thymidine solution (5 μl, 130 μCi/ml in PBS) was added, and the cells were incubated for 3 h more. The cell layers were washed three times with PBS, twice with 5% TCA, and once with ethanol/diethyl ether (3:1 vol/vol) at 0–4°C. The residues in the wells were solubilized with 0.3 M NaOH, the solution was neutralized with 6 N HCl, and radioactivity was measured in a scintillation spectrometer (Rack-Beta; Pharmacia LKB, Uppsala, Sweden).

Proteoglycan synthesis. When cultures maintained in 16-mm wells became confluent, the cells were preincubated for 24 h in 0.5 ml of MEM supplemented with 0.5% fetal bovine serum. The cells were then transferred to 0.5 ml of the same medium supplemented with PTH[1–34]. After 30 h they were exposed to 2 μCi/ml of [^{35}S]sulfate in MEM for 3 h. Proteoglycan synthesis by chondrocytes was estimated by measuring the incorporation of [^{35}S]sulfate into materials precipitated with cetylpyridinium chloride (17).

Determination of intracellular cAMP. When cultures maintained in 16-mm wells became confluent, they were preincubated for 24 h in 0.5 ml MEM with 0.5% fetal bovine serum and 60 μg/ml kanamycin. The medium was then replaced by fresh medium of the same composition, and incubation was continued for 3 h. We confirmed that this 3-h incubation did not affect the level of cAMP. PTH was added to the cultures, and the incubation was continued for 1–20 min. Then the medium was removed and the chondrocytes were overlaid with 0.5 ml of 5% TCA for 30 min at 4°C. The supernatant was then collected and used for cAMP assay. cAMP was determined by the method of Honma et al. (18) with a cAMP assay kit (Yamasa Shoyu Co.). Protein was determined by the method of Lowry et al. (19).

^{125}I-PTH binding assay and affinity labeling. ^{125}I-[Nle^8, Nle^{18}, Tyr^{34}]bovine PTH[1–34](^{125}I-PTH[1–34]) was prepared by the chloramine T method as described (20, 21). Confluent cultures grown in 16-mm wells were washed twice with binding buffer (Ham's F-12 medium supplemented with 0.2% BSA and 10 mM Hepes, pH 7.4), and exposed to ^{125}I-PTH[1–34] (50,000 cpm/well, 1,000 Ci/mmol) in 0.5 ml of binding buffer in the presence or absence of various concentrations of unlabeled PTH[1–34] at 15°C for 5 h. The solution was removed by aspiration, and the cells were washed five times with cold PBS containing 0.2% BSA. The cell monolayers were solubilized in 0.1 M NaOH containing 0.2% Triton X-100 for 30 min at room temperature, and radioactivity in the cell lysate was counted in a γ-counter (LKB).

Results

Effect of PTH on the growth of chick embryo chondrocytes in soft agar. When embryonic 17-d-old chick chondrocytes were cultured in soft agar without the addition of hormones or growth factors, no colony formation was noted (Fig. 1 *A*). PTH[1–34] increased colony formation dose-dependently with an ED_{50} of 10^{-10} M and an ED_{100} of 10^{-9} M (Table I). The efficiency of colony formation in the presence of PTH at 10^{-9} M was 12-fold that in cultures without the hormone (Table I). PTH induced the formation of loose clusters of cells (loose colonies) 14 d after cell seeding (Figs. 1 *B* and 2), whereas bFGF induced compact colonies within 4–7 d (Figs. 1 *C* and 2) (12). In loose colonies, cells were separated from one another by an abundant proteoglycan matrix that stained with Alcian blue (not shown). PTH-stimulated chondrocytes may synthesize the abundant proteoglycan matrix during their proliferation in soft agar, and this synthesis may account for the formation of loose colonies.

Effects of age on PTH stimulation of colony formation by chick and rabbit chondrocytes. Fig. 3 illustrates the effects of age on PTH stimulation of colony formation by chondrocytes. PTH[1–34] induced colony formation by cells from embryonic 13-, 15-, 17-, and 19-d-old chicken sternal cartilage. However, scarcely any stimulation of colony formation was observed with postnatal 3- to 14-d-old chicken chondrocytes. PTH[1–34] also induced colony formation by growth-plate chondrocytes from fetal 25-d-old rabbit rib cartilages, whereas it did not induce colony formation by postnatal 21-d-old rabbit chondrocytes (Fig. 4). Furthermore, PTH[1–34] increased the DNA content of fetal rabbit chondrocytes (Fig. 5) and embryonic chick chondrocytes (not shown) in soft agar dose dependently, causing half maximal stimulation at 10^{-10} M.

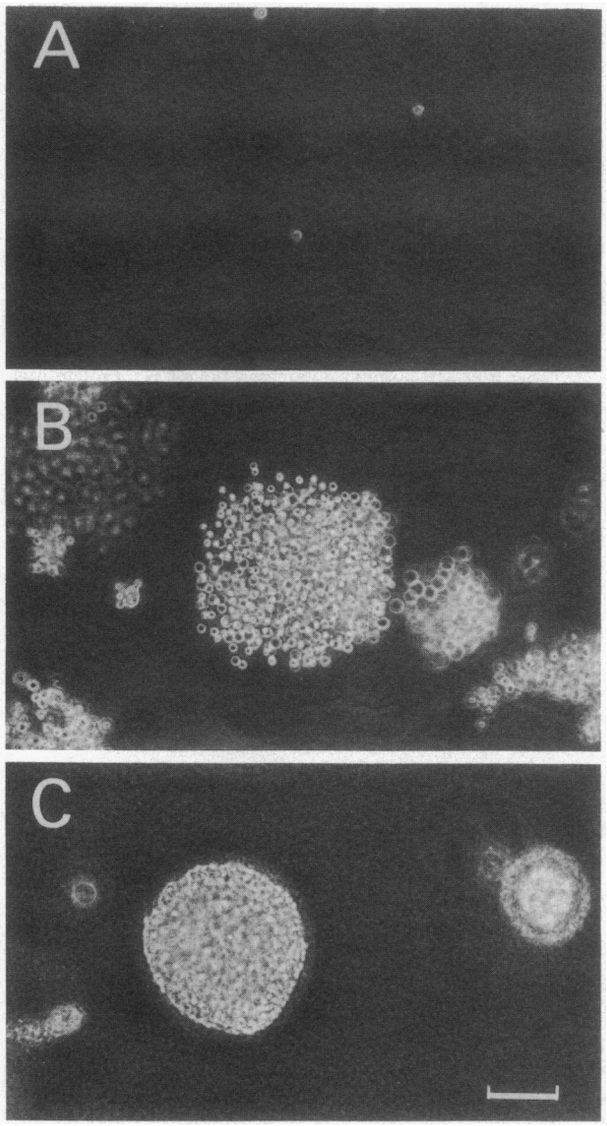

Figure 1. Morphological appearance of chondrocyte colonies in soft agar with or without PTH or bFGF. Chondrocytes isolated from embryonic 17-d-old chicken sternal cartilage were seeded (5,000 cells/0.5 ml per 16-mm well) and maintained in soft agar with no addition (*A*), with 10^{-8} M PTH[1–34] (*B*), or with 0.4 ng/ml bFGF (*C*). Pictures were taken with an Olympus phase-contrast photomicroscope on day 21. *Bar* = 100 μm.

The DNA content at the optimum dose of PTH (10^{-8} M) was 10-fold that in cultures without the hormone.

On the other hand, bFGF induced high levels of colony formation by chick and rabbit chondrocytes, irrespective of cartilage age (Figs. 3 and 4). Insulin and EGF did not induce colony formation by fetal and postnatal chondrocytes (Fig. 4).

DNA synthesis. Next we examined whether direct stimulation of DNA synthesis in chondrocytes by PTH was age dependent. In fetal 25-d-old rabbit chondrocytes, PTH[1–34] increased [^{3}H]thymidine incorporation into DNA in 24 h in a dose-dependent manner with an ED_{50} of 10^{-9} M (Fig. 6 *A*); 2.5-fold increase in [^{3}H]thymidine incorporation into DNA was observed at 10^{-8} M. In contrast, 10^{-10}–10^{-7} M PTH decreased [^{3}H]thymidine incorporation to 70–85% of the control

Table I. Effects of PTH and bFGF on Colony Formation by Chick Embryo Chondrocytes in Soft Agar

Treatment*	Compact colonies		Loose colonies	Total
	Small	Large		
None	2±1	0	10±2	13±2
PTH				
10^{-11} M	3±1	0	24±6	27±6
10^{-10} M	3±2	0	74±2	77±3
10^{-9} M	5±3	0	151±16	157±19
10^{-8} M	6±3	1±1	153±17	160±21
10^{-7} M	17±6	2±1	119±13	137±19
10^{-6} M	7±3	0	85±5	92±8
bFGF	159±13	185±15	31±5	376±32

* Chondrocytes isolated from embryonic 17-d-old chicken sternal cartilages were seeded (5,000 cells/0.5 ml per 16-mm well) and maintained in soft agar with or without various concentrations of PTH[1-34] or bFGF (0.4 ng/ml). After 21 d three types of colonies were counted: compact and small (0.1–0.2-mm-diam); compact, large colonies (>0.2 mm); and loose colonies (>40 cells). Values are averages ± SD for four cultures.

level in postnatal 21-d-old rabbit chondrocytes (Fig. 6 *B*). On the other hand, bFGF, EGF, and insulin increased [^{3}H]thymidine incorporation into DNA in both fetal and postnatal chondrocytes (Fig. 6, *A* and *B*). Of the mitogenic factors tested, only PTH had an age-dependent effect on [^{3}H]thymidine uptake in chondrocytes. Similar results were obtained in four independent experiments.

Effects of age on PTH stimulations of proteoglycan and cAMP syntheses. We also examined the effects of age on PTH stimulations of proteoglycan and cAMP syntheses. PTH[1–34] increased [^{35}S]sulfate incorporation into proteoglycans by fetal 25-d-old rabbit chondrocytes in a dose-dependent manner with an ED_{50} of ~ 10^{-8} M and an ED_{100} of 10^{-7} M (Fig. 7). The PTH stimulation of [^{35}S]sulfate incorporation into proteoglycans in fetal chondrocytes was similar to that of postnatal 28-d-old chondrocytes as shown in Fig. 7 and in other replicate experiments (data not shown).

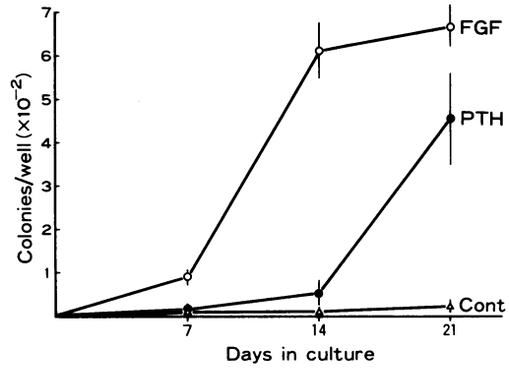

Figure 2. Time courses of PTH- and bFGF-inductions of colony formation by chick embryo chondrocytes. Embryonic 17-d-old chicken sternal chondrocytes were seeded (5,000 cells/0.5 ml per 16-mm well) and maintained in soft agar with no addition (△), with 10^{-8} M PTH[1–34] (●), or with 0.4 ng/ml bFGF (○). Points and bars are averages±SD for four cultures.

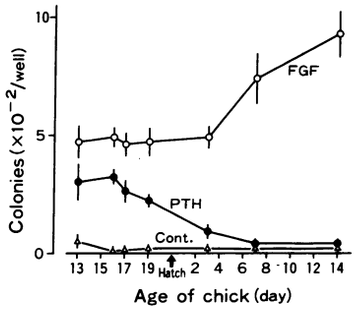

Figure 3. Effects of age on PTH and bFGF stimulations of colony formation by chick chondrocytes. Cells were isolated from embryonic or postnatal chicken sterna on the indicated days before or after hatching. The cells were seeded (5,000 cells/0.5 ml per 16-mm well) and maintained in soft agar in the presence (●) or absence (△) of 10^{-8} M PTH[1–34], or 0.4 ng/ml bFGF (○). After 21 d colonies were counted. Values are averages±SD for four cultures.

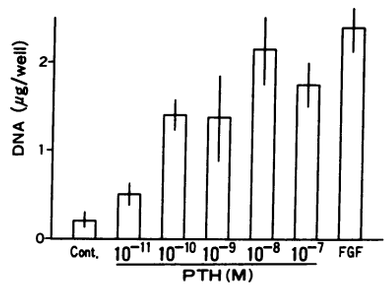

Figure 5. DNA contents of cultures of fetal rabbit growth plate in soft agar with various concentrations of PTH[1–34] or with bFGF (0.4 ng/ml). Fetal rabbit chondrocytes were maintained in soft agar for 21 d as described in Fig. 4. Bars and lines are averages±SD for three cultures.

Fig. 8 shows the dose response and time course of the effect of PTH on the intracellular cAMP level in rabbit chondrocytes in monolayer cultures. PTH[1–34] increased the cAMP level in chondrocytes within 1 min in a dose-dependent manner with an ED_{50} of 10^{-8} M and an ED_{100} of 10^{-7} M. The cAMP response of fetal chondrocytes was indistinguishable from that of postnatal chondrocytes.

Effects of age on PTH receptors. Fig. 9 shows the bindings of ^{125}I-PTH[1–34] to rabbit chondrocytes in confluent cultures. In the period between fetal day 25 and postnatal day 28, the number and dissociation constant of PTH receptors ranged between 3.6 and 6.0 × 10^4 per cell, and between 0.6 and 1.1 nM, respectively. No decrease in number or affinity of receptors was observed after birth.

Discussion

PTH has been shown to increase [^{3}H]thymidine uptake by chondroprogenitor cells in organ cultures of neonatal mouse condyle (22). It also induced a 1.5-fold increase in the DNA content of chick embryo cartilage in organ culture (8, 9). However, cartilage and condyle in organ culture may include other cell types besides chondrocytes, such as perichondrium cells and chondroclasts, and the presence of these cells, as well as individual differences between animals, may affect results on the effect of PTH on chondrocytes in organ culture. Thus, further studies using well-characterized chondrocyte cultures were necessary to determine details of the mitogenic effect of PTH.

In the present study we examined the effect of PTH on growth of rabbit and chick chondrocytes in soft agar and in monolayer cultures as a function of age. Our results showed that PTH caused a 12- to 15-fold increase in colony formation by embryonic rabbit growth-plate chondrocytes and chick sternal chondrocytes in soft agar with a 10-fold increase of their DNA. PTH also caused 2.5-fold increase in [^{3}H]thymidine incorporation into DNA in fetal rabbit chondrocytes in monolayer cultures. These results provide evidence that PTH is a potent mitogen for both mammalian and avian chondrocytes in the absence of other cell types. The growth-promoting effect of PTH was specific for overtly differentiated chondrocytes from embryos. No growth stimulation was observed with postnatal chondrocytes, embryonic fibroblasts, or dedifferentiated chondrocytes, which had been passaged at low density on plastic dishes or preincubated with 5-bromodeoxyuridine or retinoic acid (data not shown).

Although its stimulation of chondrocyte colony formation was striking, PTH caused only a 2.5-fold stimulation of [^{3}H]-

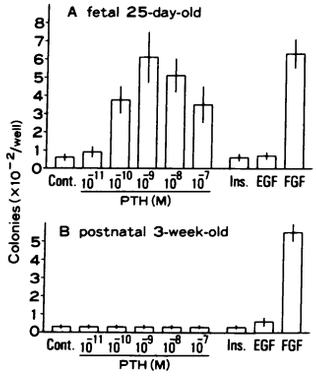

Figure 4. Effects of PTH and various mitogenic factors on colony formation by fetal and postnatal rabbit chondrocytes. Chondrocytes were isolated from rib cartilages of fetal 25-d-old (*A*) or postnatal 21-d-old (*B*) rabbits. Cells were seeded (5,000 cells/0.5 ml per 16-mm well) and maintained in soft agar with or without 10^{-11}–10^{-7} M PTH, 1 μg/ml of insulin, 30 ng/ml EGF, or 0.4 ng/ml bFGF. After 21 d colonies were counted. In cultures of fetal chondrocytes, loose and compact colonies were formed in PTH, whereas only compact colonies were formed in bFGF. Bars and vertical lines are averages±SD for four cultures.

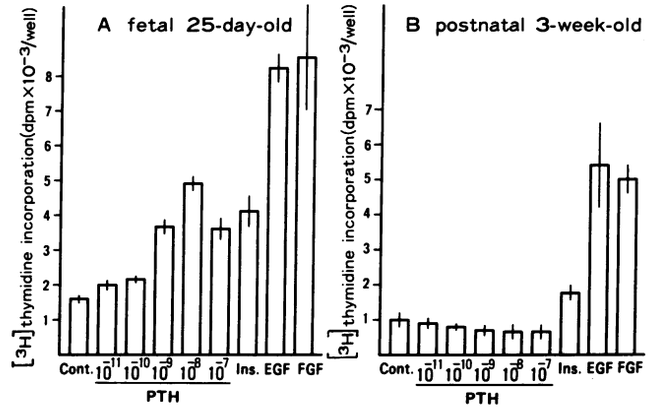

Figure 6. Effects of PTH, insulin, EGF, and bFGF on DNA synthesis in fetal and postnatal rabbit chondrocytes in monolayer cultures. Chondrocytes were isolated from rib cartilage of fetal 25-d-old (*A*) or postnatal 21-d-old (*B*) rabbits. The cells were grown to confluency in plastic wells in 0.5 ml MEM with 10% fetal bovine serum and antibiotics. The cells were then exposed to MEM with 0.5% fetal bovine serum in the presence or absence of 10^{-11}–10^{-7} M PTH, 1 μg/ml insulin, 30 ng/ml EGF, or 0.4 ng/ml bFGF for 27 h. Bars and lines are averages±SD for four wells. Similar results were obtained in four independent series of studies. The inhibition of [^{3}H]thymidine incorporation by PTH in postnatal chondrocytes was statistically significant ($P < 0.01$).

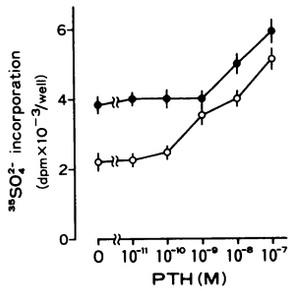

Figure 7. Effect of PTH on [^{35}S]-sulfate incorporation into proteoglycans by fetal 25-d-old (○) and postnatal 28-d-old (●) rabbit chondrocytes. Chondrocytes were seeded and maintained in 16-mm microwells as described for Fig. 6. Confluent cultures were exposed for 30 h to the indicated concentrations of PTH[1–34] in 0.5 ml of MEM with 0.5% fetal bovine serum. Bars and lines are averages±SD for three cultures.

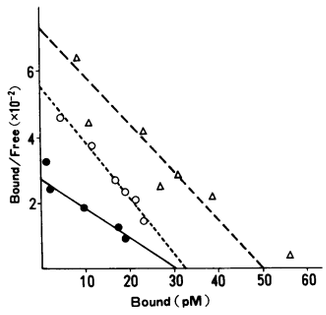

Figure 9. Equilibrium saturation binding of radioiodinated [Nle^8,Nle^{18},Tyr^{34}]bPTH[1–34] in rabbit chondrocytes. Chondrocytes were isolated from the growth plates of fetal 25-d-old (●), postnatal 4-d-old (○), and 28-d-old (△) rabbits, seeded in 16-mm plastic wells, and grown to confluency in medium with 10% serum. Confluent cells were incubated with increasing concentrations of radioiodinated [Nle^8,Nle^{18}, Tyr^{34}]bPTH[1–34] as described in Methods. Both total and nonspecific binding were determined in duplicate or triplicate; the divergence of triplicate or duplicate values was 10% or less. Nonspecific binding was determined in the presence of unlabeled ligands (3×10^{-7} M). The amount of ^{125}I-[Nle^8,Nle^{18},Tyr^{34}]bPTH[1–34] was then determined. Scatchard plots of data are shown. Experiments were done twice with similar results.

thymidine uptake, whereas bFGF and EGF caused a 5- to 10-fold stimulation. Moreover, there was a long lag time before induction of colony formation by PTH, whereas bFGF had a rapid effect on colony formation. These observations suggest that PTH induces production of a growth factor(s) by chondrocytes. Previous studies have shown that PTH increases the synthesis of insulin-like growth factor 1 (IGF-1) by bone cells (23). Some growth factors are known to be synthesized by chondrocytes; namely, IGFs (24–27), transforming growth factor-beta (TGF-beta) (28), and cartilage-derived factor (17, 25). TGF-beta increases the efficiency in colony formation by chick embryo chondrocytes in the presence of bFGF, although TGF-beta alone has little effect on the colony formation by chondrocytes (14). A 26-kD cartilage-derived factor also induces colony formation by chick embryo chondrocytes (Kato, Y., unpublished observations). However, IGF-1 has little effect on colony formation by chick embryo chondrocytes in the presence (not shown) or absence of PTH (12), probably because of the presence of 10% serum in soft agar. Thus, it is unlikely that the mitogenic action of PTH is mediated by the increase in IGF-1 synthesis, although PTH does increase threefold the production of IGF-1 by embryonic and postnatal rabbit chondrocytes in monolayer cultures (Iwamoto, M., and Y. Kato, unpublished observations). In addition, PTH may stimulate colony formation by chondrocytes in soft agar by increasing synthesis of the extracellular matrix, because a cartilage matrix is required as an anchoring support for growth in semiliquid medium (12).

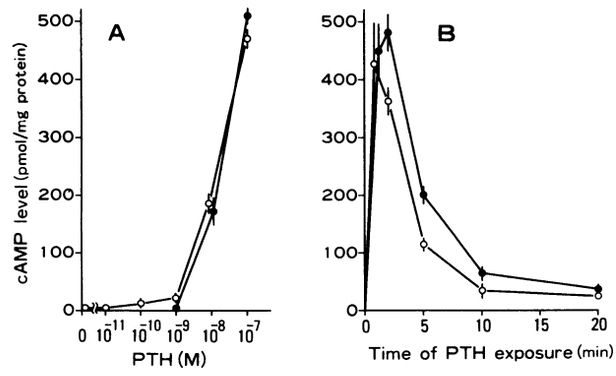

Figure 8. Effect of PTH on the intracellular concentration of cAMP in fetal 25-d-old (○) and postnatal 28-d-old (●) rabbit chondrocytes. *A*, PTH[1–34](10^{-11}–10^{-7} M) was added to confluent cultures of rabbit chondrocytes, and cAMP was extracted at 5 min. *B*, PTH[1–34] (10^{-7} M) was added to confluent cultures, and cAMP was extracted at the indicated times after its addition.

In this study we found that the number and affinity of PTH receptors in chondrocytes do not decrease after birth. Cross-linking of ^{125}I-PTH to fetal and postnatal chondrocytes revealed the presence of 72-kD PTH receptors (Kato, Y., and M. Iwamoto, unpublished observations). The receptors are functional in postnatal chondrocytes because PTH induced proteoglycan and cAMP responses by these cells. We found in the present study that bFGF, EGF, and insulin enhance [^{3}H]thymidine uptake by chondrocytes, irrespective of cartilage age. These observations suggest that a signaling pathway for growth in response to PTH selectively becomes inoperative at postreceptor levels after birth. This is of interest because hormone responsiveness is usually determined by the hormone and receptor levels.

Although PTH stimulated both proliferation and the synthesis of proteoglycans and cAMP in fetal chondrocytes, the concentrations of PTH required for stimulations of proteoglycan and cAMP synthesis were 100-fold higher than those required for the stimulation of proliferation. This finding indicates that PTH stimulation of chondrocyte phenotypic expression is not linked to its growth stimulation, and that the mitogenic action of PTH is not mediated by cAMP. The effects of cAMP analogue mimicked those of PTH in chondrocytes (5, 8, 9, 29, 30), but dibutyryl cAMP enhanced chondrocyte growth (8, 9, 30) in an age-independent fashion (Kato, Y., and T. Koike, unpublished observations). Long-acting cAMP analogues may cause abnormal phosphorylation through sustained activation of cAMP-dependent protein kinases (see reference 4).

The physiological significance of the in vitro actions of PTH on chondrocytes is unknown. However, human fetal parathyroid glands synthesize PTH as early as 12–13 wk of gestation in vitro (31), although PTH does not cross the placenta (32). Furthermore, PTH is found in fetal blood of various animals, and its level (10^{-11}–10^{-10} M) is similar to the maternal level (monkey [33]; miniature pig [34]; rabbit [Kato, Y., and T. Koike, unpublished observations]). Thus, PTH may participate in vivo in supporting rapid growth of cartilage in fetal life.

In conclusion, in addition to being a regulator of calcium homeostasis, PTH was shown in this study to be a potent mitogen for chondrocytes in embryos. The abrupt disappear-

ance of the mitogenic action of PTH observed after birth may play a role in the control of skeletal growth in neonatal life.

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