

Intermittent Parathyroid Hormone Treatment Can Promote Linear Growth in the Ovariectomized Growing Rat

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Abstract

To compare the effect of intermittent parathyroid hormone (PTH) treatment with that of estrogen treatment on epiphyseal growth in ovariectomized rats, 46 Sprague-Dawley female rats aged 9-10 weeks (about 200-220 g) were either ovariectomized or sham operated. From 6 weeks after ovariectomy (ovx), rats were daily injected with subcutaneous human recombinant PTH (1-84)-dosed 30 $\mu\text{g}/\text{kg}$ (the low dose PTH-treated group) or 300 $\mu\text{g}/\text{kg}$ (the high dose PTH-treated group), 17 β -estradiol (the 17 β -estradiol-treated group, 30 $\mu\text{g}/\text{kg}$) or vehicle (the ovx-alone group), 5 times a week for 4 weeks. The decalcified sections of the distal femoral epiphyseal plate were analyzed on light microscopy after H&E stain, and the lengths of the zones of proliferation, maturing and hypertrophic chondrocytes were measured. The length of the growth plate, the zone of proliferation and the zone of hypertrophic chondrocyte in the ovx-alone group were significantly shorter than those of the sham-operated group. The treatment of 17 β -estradiol speeded up the differentiation of cells from proliferating chondrocytes to maturing and hypertrophic chondrocytes even though the length of the growth plate was comparable to that of the sham-operated group. Both low and high dose PTH treatments increased the length of the growth plate, and those lengths were comparable to that of the sham-operated group. The fractions of proliferating, maturing and hypertrophic zone in the low dose PTH-treated group were also comparable to those of the sham-operated group. However, high dose PTH treatment slowed down the differentiation of cells from proliferating chondrocytes to maturing and hypertrophic chondrocytes to a greater extent, and therefore the fraction of proliferating chondrocytes of the high dose PTH-treated group was larger than that of the low dose PTH-treated group (73.8 ± 1.8 Vs $63.3 \pm 1.3\%$, $p < 0.005$). From these results, we showed that intermittent PTH treatment could promote linear growth in the ovariectomized growing rat. We propose that PTH may be an alternative drug candidate for promoting linear growth of long bones without the risk for early closure of the growth plate.

Key Words: Parathyroid hormone, 17-estradiol, growth plate, linear growth

INTRODUCTION

The regulation of postnatal somatic growth is complex. Genetic, nutritional factors and hormones exert regulatory functions.¹ Alterations in growth hormone pulse amplitude acting synergistically with sex steroids result in the growth spurt of puberty.² Growth hormone and insulin-like growth factor-1 (IGF-1) have different target cells in the epiphyseal growth plate.² While growth hormone promotes

mainly the growth of long bones by stimulating the slowly dividing prechondrocytes in the germinative cell layer, IGF-I promotes clonal expansion in the proliferative cell layer of growth hormone primed cells. Hypogonadism in children could result in the attenuation of linear growth without administration of sex hormones.³ In patients with estrogen deficiency such as Turner's syndrome, low dose 17 β -estradiol therapy for growth promotion is not effective in increasing the final adult height and could, in fact, accelerate bone aging to an extent that might compromise the adult height.^{4,5} However, the action mechanism of sex steroids on the growth plate has not been fully elucidated. Recently, several groups have begun studies on how local factors influence the overall patterning of bone tissue and the differentiation of cells on the growth plate.^{6,7}

Parathyroid hormone related peptide (PTHrP) has been known to cause humoral hypercalcemia of a malignancy, but it is a growth factor in its own

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right.⁸ The chondrocyte columns of the growth plate in PTHrP or Parathyroid hormone (PTH)/PTHrP receptor knock-out mice were much shorter than those in normal mice.^{5,9} The expression of a constitutively active mutant PTH-PTHrP receptor in Jansen-type metaphyseal chondrodysplasia led to the abnormal formation of endochondral bone in this rare form of short-limbed dwarfism.¹⁰ All the above findings suggest that one of the important biological roles of PTHrP lies in the regulation of normal skeletal development by modulating the proliferation and differentiation of chondrocytes.

Recently, PTH-2 receptor was cloned in the brain and is also known to be expressed in the pancreas, testis and placenta.¹¹ In many tissues including bone, kidney and cartilage, however, PTH and PTHrP activate the same PTH/PTHrP receptor-1.¹² In growing long bones, PTH/PTHrP receptor-1 is expressed abundantly in cells of the transitional zone between proliferating and hypertrophic chondrocytes.¹³ While continuous PTH treatment increases bone resorption, intermittent PTH treatment exerts an anabolic effect on trabecular bone, also increasing the trabecular connectivity.^{14,15} Therefore, we hypothesize that intermittent PTH treatment may have a positive

effect on promoting linear growth by activating the PTH/PTHrP receptor in some children of short stature.

In this study, we have attempted to clarify the following questions in the ovariectomized growing rat: 1) Does ovariectomy have any effects on epiphyseal growth?; 2) How does 17-estradiol function on epiphyseal growth?; 3) Can intermittent PTH treatment promote the linear growth?; 4) Are the effects of PTH on the growth plate different from those of 17 β -estradiol?; 5) Are there any differences between low and high dose PTH treatment in its effect on epiphyseal growth?

MATERIALS AND METHODS

We used 46 female Sprague-Dawley rats approximately 9–10 weeks of growing age and weighing from 200 to 220 gm at the beginning of the experiment. 17 β -estradiol was purchased from Sigma Chemical Co. (St. Louis, MO, U.S.A.) and recombinant human PTH (1–84) was kindly donated by the Korea Green Cross Corporation (Seoul). The rats were divided into two groups, 35 test animals and

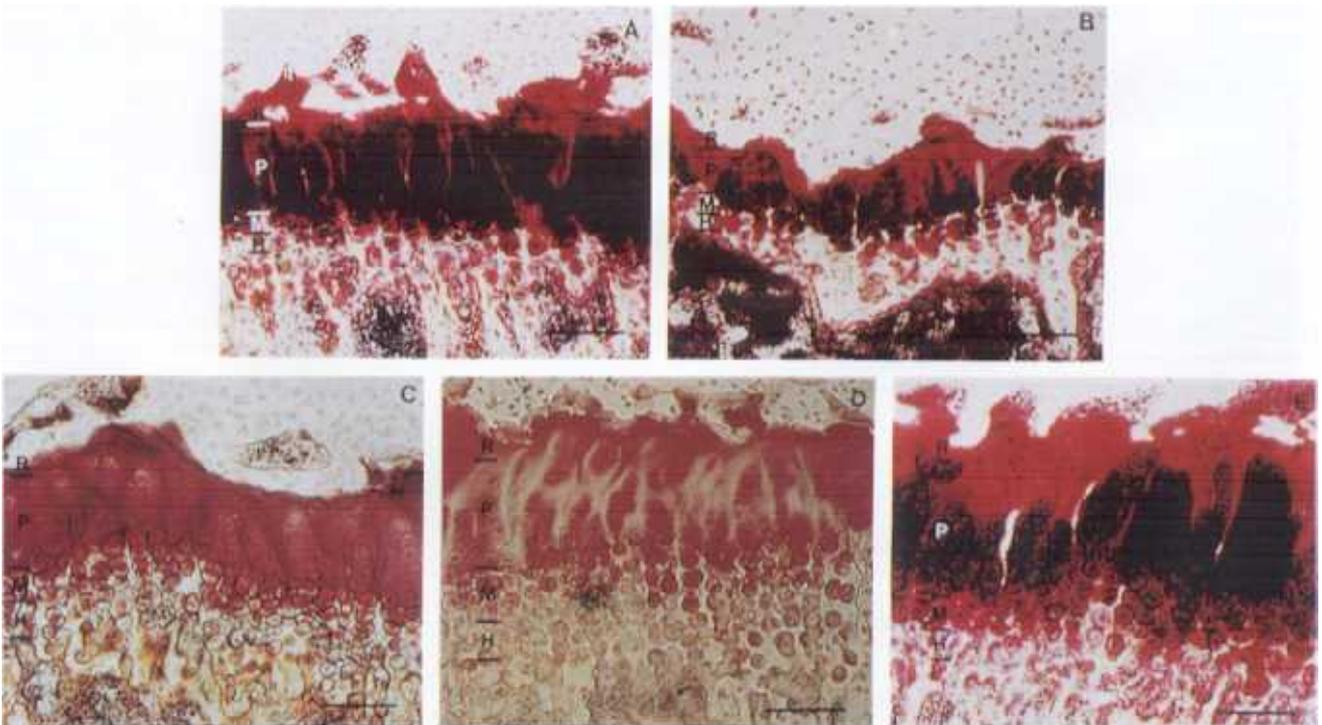


Fig. 1. Longitudinal section through the distal epiphyseal plate of tibia. The epiphyseal plate is divided into four zones: resting (R), proliferating (P), maturing (M), and hypertrophic (H) chondrocytes. (A) the sham-operated group, (B) the ovx-alone group, (C) the 17 β -estradiol-treated group, (D) the low dose PTH-treated group, (E) the high dose PTH-treated group. Decalcified, H-E stain (magnification, $\times 100$), bar = 100 μ m.

11 controls. All rats were fed *ad libitum* and had free access to water. The control and test groups underwent sham operation and ovariectomy, respectively. Six weeks after operation, the control group (the sham-operated group, $n=11$) was injected daily with subcutaneous placebo, whereas the test group was subdivided into groups of 4 and given: 1) vehicle in the Ovariectomy (OVX) alone (the OVX-alone group, $n=9$); 2) a dose of $30 \mu\text{g}$ of 17β -estradiol/kg of body weight (the 17β -estradiol-treated group, $n=10$); 3) a low dose of recombinant hPTH (1-84) ($30 \mu\text{g}$ /kg of body weight (the low dose PTH-treated group, $n=8$); 4) a high dose of recombinant hPTH (1-84) ($300 \mu\text{g}$ /kg of body weight (the high dose PTH-treated group, $n=8$), respectively. The rats were sacrificed four weeks after treatment. The entire femoral bones were removed and fixed in 5% formalin solution. Paraffin sections after decalcification were prepared in the usual way and stained with hematoxylin and eosin (H&E). Only the distal epiphyseal-metaphyseal growth plate in mid-sagittal section was examined microscopically to study the relationship among the four zones of chondrocytes in terms of the length of each zone: resting, proliferating, maturing and hypertrophic zone^{16,17} (Fig. 1). The length of each zone was measured by using the reference bar of $100 \mu\text{m}$ in the microscopic image. The margin of each zone was determined by the pathologist with special reference to the morphologic characteristics of each zone reported previously.^{16,17} The fraction of each zone was calculated as follows:

$$\frac{\text{The length of each zone}}{\text{The total length of the growth plate}} \times 100(\%)$$

The research ethics committee of Severance Hospital, Yonsei University, approved the study.

Statistics

Statistical comparison of the 5 groups was performed by means of Kruskal-Wallis test. P value of <0.05 was considered statistically significant.

RESULTS

The total length of the growth plate

The total length of the growth plate was the shortest in the ovx-alone group ($133.3 \pm 9.6 \mu\text{m}$)

(Fig. 2). Compared to the ovx-alone group, the total length increased significantly in the 17β -estradiol, the low dose PTH and the high dose PTH treated groups ($215.4 \pm 9.6 \mu\text{m}$, $268 \pm 15.2 \mu\text{m}$ and $237.5 \pm 19.3 \mu\text{m}$, respectively). However, the total length in each of these groups was not significantly increased compared to the sham-operated group ($227.1 \pm 17.6 \mu\text{m}$) and there were no significant differences among the 17β -estradiol, the low dose PTH and the high dose PTH-treated groups.

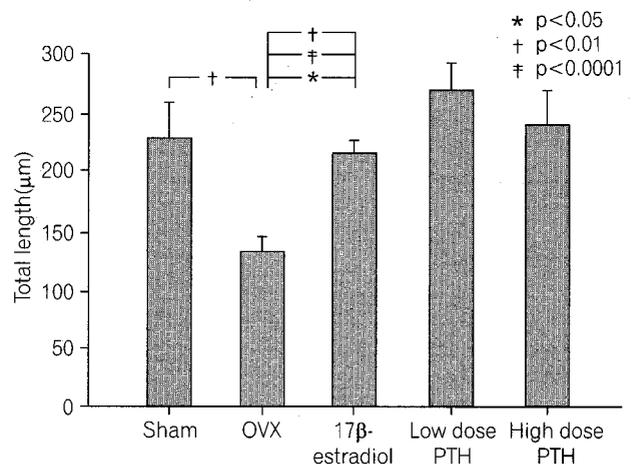


Fig. 2. The total length of the growth plate in the sham-operated group, the ovx alone-group, the 17β -estradiol-treated group, the low dose PTH (1-84)-treated group and the high dose PTH (1-84)-treated group. * $p < 0.05$, † $p < 0.01$, ‡ $p < 0.0001$.

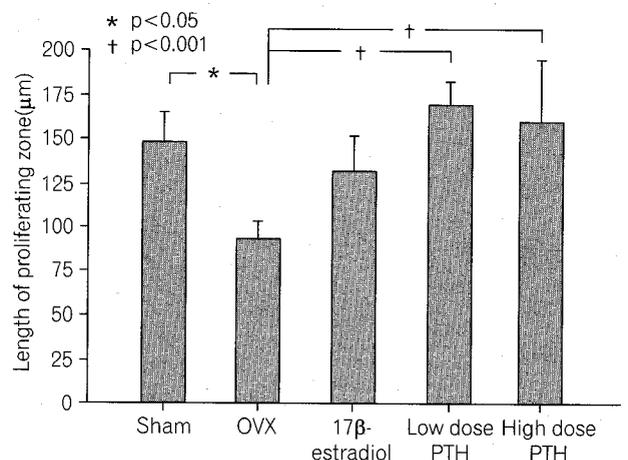


Fig. 3. The length of zone of proliferating chondrocytes in the sham-operated group, the ovx-alone group, the 17β -estradiol-treated group, the low dose PTH (1-84)-treated group and the high dose PTH (1-84)-treated group. * $p < 0.05$, † $p < 0.001$.

The length of zone of proliferating chondrocytes

The length of the zone of proliferating chondrocytes in the ovx-alone group ($94.3 \pm 6.8 \mu\text{m}$) was shorter than those in the sham-operated ($147.5 \pm 10.7 \mu\text{m}$), the low dose PTH ($169.4 \pm 9.0 \mu\text{m}$) and the high dose PTH ($174.1 \pm 12.3 \mu\text{m}$) groups (Fig. 3). While 17β -estradiol treatment after ovariectomy did not increase the length of zone of proliferating chondrocytes, low and high dose PTH treatment after ovx significantly increased the length of the zone of proliferating chondrocytes ($p < 0.05$ and $p < 0.001$,

respectively). However, there was no difference in this length between the low dose and the high dose PTH-treated groups.

The length of zone of maturing chondrocytes

Compared to the length of zone of maturing chondrocytes in the ovx-alone group ($20.3 \pm 2.4 \mu\text{m}$), it was longer in the 17β -estradiol and the low dose PTH-treated groups ($46.4 \pm 5.5 \mu\text{m}$ and $49.9 \pm 3.4 \mu\text{m}$, respectively) (Fig. 4). However, the length in the high dose PTH-treated group was no longer than that of the ovx-alone group ($37.0 \pm 4.3 \mu\text{m}$ Vs $20.3 \pm 2.4 \mu\text{m}$).

The length of zone of hypertrophic chondrocytes

Compared to the sham-operated group ($42.3 \pm 4.0 \mu\text{m}$), the length of hypertrophic chondrocytes was shorter in the ovx-alone group ($18.7 \pm 2.4 \mu\text{m}$) (Fig. 5). 17β -estradiol and low dose PTH treatment after ovariectomy increased the length of hypertrophic chondrocytes significantly ($37.6 \pm 4.6 \mu\text{m}$ and $49.3 \pm 4.7 \mu\text{m}$, respectively). However, high dose PTH treatment did not increase the length of hypertrophic chondrocytes ($26.4 \pm 4.8 \mu\text{m}$). Compared to the low dose PTH-treated group, the length of hypertrophic chondrocytes was significantly lower in the high dose PTH-treated group ($49.3 \pm 4.7 \mu\text{m}$ Vs $26.4 \pm 4.8 \mu\text{m}$).

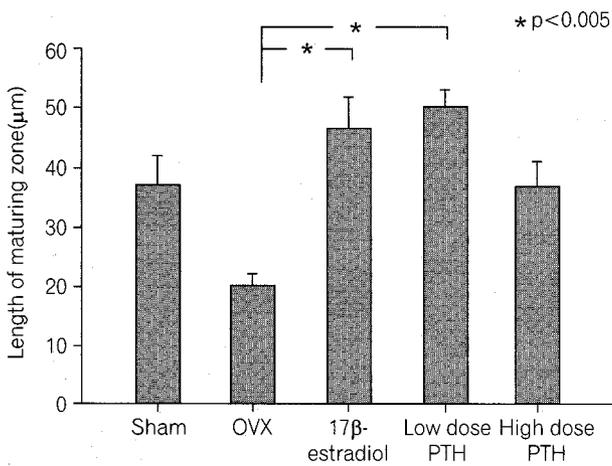


Fig. 4. The length of zone of maturing chondrocytes in the sham-operated group, the ovx-alone group, the 17β -estradiol-treated group, the low dose PTH (1-84)-treated group and the high dose PTH (1-84)-treated group. * $p < 0.005$.

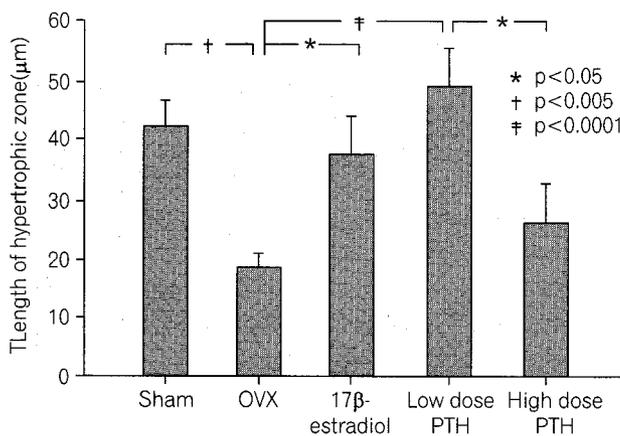


Fig. 5. The length of zone of hypertrophic chondrocytes in the sham-operated group, the ovx-alone group, the 17β -estradiol-treated group, the low dose PTH (1-84)-treated group and the high dose PTH (1-84)-treated group. * $p < 0.05$, † $p < 0.005$, ‡ $p < 0.0001$.

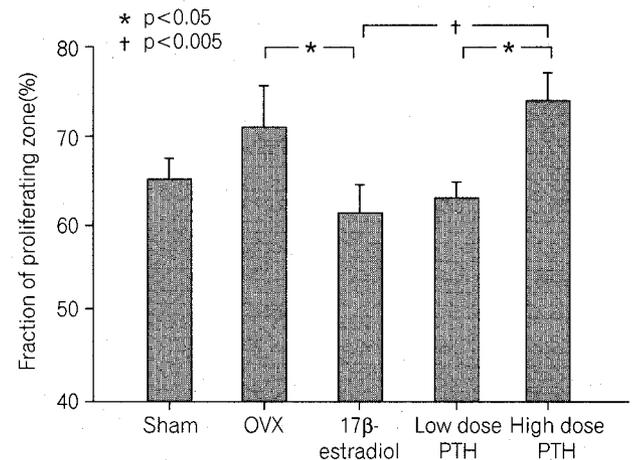


Fig. 6. The fraction of proliferating zone on the growth plate in the sham-operated group, the ovx-alone group, the 17β -estradiol-treated group, the low dose PTH (1-84)-treated group and the high dose PTH (1-84)-treated group. * $p < 0.05$, † $p < 0.005$.

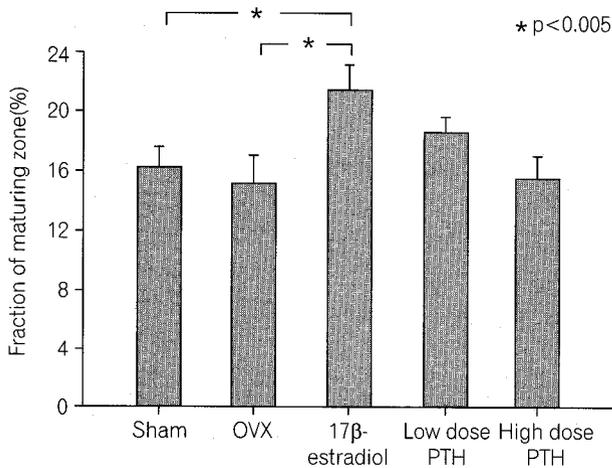


Fig. 7. The fraction of maturing zone on the growth plate in the sham-operated group, the ovx-alone group, the 17 β -estradiol-treated group, the low dose PTH (1-84)-treated group and the high dose PTH (1-84)-treated group. * $p < 0.05$.

The fraction (%) of the zone of proliferating chondrocytes in the growth plate

17 β -estradiol treatment after ovariectomy decreased the fraction of the proliferating zone on the growth plate significantly (ovx-alone group; $71.0 \pm 2.2\%$, 17 β -estradiol-treated group; $61.4 \pm 1.8\%$, $p < 0.05$) (Fig. 6). The fraction of the proliferating zone on the growth plate in high dose PTH treatment after ovariectomy increased significantly and it was larger than those of the 17 β -estradiol ($73.8 \pm 1.8\%$ Vs $61.4 \pm 1.8\%$, $p < 0.05$) and the low dose PTH-treated group ($73.8 \pm 1.8\%$ Vs $63.3 \pm 1.3\%$, $p < 0.005$). Compared to the sham-operated group ($65.3 \pm 1.6\%$), however, there was no increment of this value in the 17 β -estradiol, the low dose PTH and the high dose PTH-treated groups.

The fraction (%) of the maturing zone in the growth plate

17 β -estradiol treatment after ovariectomy increased the fraction of the maturing zone on the growth plate significantly (the ovx-alone group; $15.2 \pm 1.3\%$, the 17 β -estradiol-treated group; $21.4 \pm 1.4\%$, $p < 0.05$) (Fig. 7). The fraction of the maturing zone on the growth plate in the 17-estradiol-treated group was also larger than that in the sham-operated group ($16.1 \pm 1.1\%$, $p < 0.05$). However, PTH treatment did not change the value significantly.

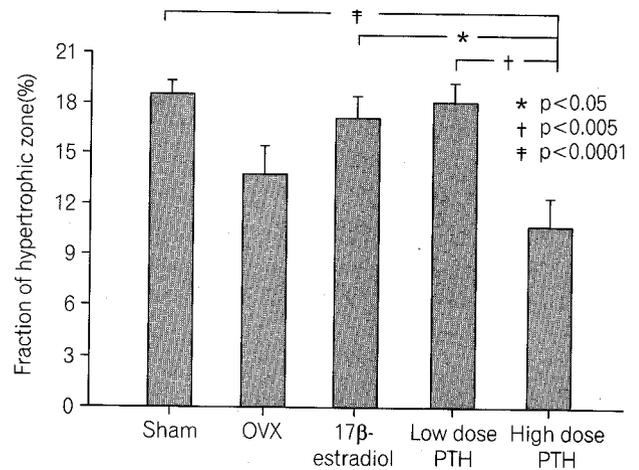


Fig. 8. The fraction of hypertrophic zone on the growth plate in the sham-operated group, the ovx-alone group, the 17 β -estradiol-treated group, the low dose PTH (1-84)-treated group and the high dose PTH (1-84)-treated group. * $p < 0.05$, † $p < 0.005$, ‡ $p < 0.001$.

The fraction (%) of the zone of hypertrophic chondrocytes in the growth plate

There was no difference in the fraction of the zone of hypertrophic chondrocytes on the growth plate among the sham-operated, the ovx-alone, the 17 β -estradiol and the low dose PTH-treated groups ($18.5 \pm 0.8\%$, $13.8 \pm 1.4\%$, $17.1 \pm 0.9\%$ and $18.1 \pm 1.0\%$, respectively) (Fig. 8). If we compare the fraction of the zone of hypertrophic chondrocytes on the growth plate in the sham-operated, the 17 β -estradiol and the low dose PTH-treated groups, it was significantly lower in the high dose PTH-treated group ($10.7 \pm 1.4\%$).

DISCUSSION

Growth plate chondrocytes play a pivotal role in promoting bone growth. However, the cellular mechanisms exerting their influence have only been clarified in a few well-characterized growth promoting substances.¹⁸ In this study, we showed clearly that intermittent PTH treatment can promote linear growth, and the response to 17 β -estradiol on the growth plate was different from that of parathyroid hormone in the ovariectomized growing rat. Our findings may aid in understanding the mechanism of epiphyseal growth and also help to develop a new potential drug for short stature.

Hypogonadism in children invariably results in

attenuation of linear growth once the bone age reaches about 10–12 years, unless sex hormones are administered.² Our study also confirmed that the total length of the epiphyseal plate was markedly shortened in ovariectomized growing rats. Compared to the sham-operated group, the significant differences in the ovx-alone group were shortening of the zone of proliferating chondrocytes and hypertrophic chondrocytes. In patients with true precocious puberty, the sustained increase in sex steroid leads to the advancement of skeletal age, a pubertal growth spurt, and a temporary greater than average height compared with normal age-matched children.⁴ In this study, short-term treatment with 17 β -estradiol enlarged the zone of maturing chondrocytes and hypertrophic chondrocytes, while the length of the growth plate was comparable to that of the sham-operated group. In other words, the fraction of maturation on the growth plate increased while that of proliferating chondrocytes decreased significantly. These findings may suggest a role of 17 β -estradiol treatment in speeding up the differentiation of cells from proliferating chondrocytes to their mature form following reduction of the proliferating chondrocyte pool, thereby leading to earlier closure of the growth plate.

Several types of PTH receptor have been identified.¹² The first receptor, which has been cloned and well characterized, is "PTH/PTHrP receptor-1". It is activated not only by PTH, but also by PTHrP.¹⁹ Recently, Kronenberg et al., after careful observation of mice missing either the PTHrP or the PTH/PTHrP receptor gene, proposed that PTHrP controls the pace of differentiation in the growth plates and that the Indian hedgehog (*Ihh*)-PTHrP feedback system regulated the rate of chondrocyte differentiation, balancing the growth and ossification of long bones.²⁰ According to their hypothesis, as proliferating chondrocytes are destined to undergo hypertrophy, they express high levels of PTH/PTHrP receptor. When they subsequently become committed to this pathway, they transiently express *Ihh*, until they later become fully hypertrophic.^{5,20} Indian hedgehog, made by pre-hypertrophic and early hypertrophic chondrocyte, acts on perichondrial cells to increase the levels of PTHrP mRNA.^{5,20} We took advantage of their proposal and hypothesized that systemic PTH treatment, not locally-produced PTHrP, could also mimic this effect of PTHrP on the pace of differentiation in the growth plate.

As we anticipated, intermittent PTH treatment after ovariectomy increased the total length of the

growth plate significantly, and the length was comparable to that of the sham-operated group. Lindsay et al. observed that low dose-intermittent PTH (1–34) injection in postmenopausal women increased the serum PTH (1–34) level with variable peak levels averaging 10 times normal, and that the peptide was cleared from the circulation with a mean $t_{1/2}$ of 75 min. Peptide administration was followed by an immediate decline in endogenous secretion of PTH (1–84) and it remained suppressed at about 65% of the basal value for 4 hours.^{21,22} A similar pharmacokinetic change of serum PTH after hPTH (1–84) injection in rat might influence the fine tuning of the local *Ihh*-PTHrP feedback system and slow the differentiation of proliferating chondrocytes to hypertrophic chondrocytes transiently, following the increment of the total length of the growth plate without early closure.

There was some dose-effectiveness relationship in PTH treatment. The most striking differences between the low dose and high dose PTH-treated groups were observed in the fraction of proliferating chondrocytes, and the length of zones of maturing and hypertrophic chondrocytes on the growth plate. High dose PTH treatment more efficiently slowed the differentiation of cells from proliferating chondrocytes to maturing and hypertrophic chondrocytes. Therefore, the fraction of proliferating chondrocytes on the growth plate increased greatly, even though the total length of the growth plate was no different from that of the low dose PTH-treated group. By contrast, the low dose PTH treated-group showed a balanced increase in the fraction of proliferating, maturing and hypertrophic chondrocytes: the fractions of each zone in the low dose PTH-treated group were similar to those in the sham-operated group.

The mechanism of promoting linear growth by intermittent PTH treatment was different from that in 17 β -estradiol. 17 β -estradiol treatment after ovariectomy speeded up the differentiation of cells from proliferating chondrocytes to mature chondrocytes, followed by an accelerated exhaustion of proliferating chondrocytes. In this study, however, we could not demonstrate the precise mechanisms by which such effects of 17 β -estradiol are delivered to the growth plate. One of several plausible explanations is that 17 β -estradiol may also modulate the fine-tuning of the local *Ihh*-PTHrP feedback system. The temporal decline in uterine PTH/PTHrP receptor mRNA levels was observed 2 and 4 hours after 17 β -estradiol treatment.²³ However, there have been no reports yet

as to whether 17 β -estradiol changes the expression of *Ibb*, PTHrP or PTH/PTHrP receptor in the cells of the growth plate. We need more studies on this issue. By contrast, daily single injection of PTH promoted the growth of long bone by transient inhibition of the differentiation of cells from proliferating chondrocytes to hypertrophic chondrocytes, and there was no evidence of accelerated reduction of proliferating chondrocytes. Our observations warrant notice in respect to promoting the linear growth of long bones by handling of the fine-tuning of the local *Ibb*-PTHrP feedback loop with daily single subcutaneous injection of PTH.

In conclusion, intermittent PTH treatment in ovariectomized growing rats can promote linear growth without any risk for early closure of the growth plate. Since therapeutic interventions, including growth hormone, fail to bring about a catch-up growth to the mean height of the general population in some children with familial constitutional short stature or Turner's syndrome, PTH could be an alternative drug candidate for promoting linear growth of long bones in these children.

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