INVESTIGATIVE NUCLEAR MEDICINE

Effect of Hypophysectomy and Hormonal Replacement on the Uptake of Tc-99m Methylene Diphosphonate in the Metaphysis and Shaft of the Rat Femur: Concise Communication

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We have investigated the uptake of Tc-99m methylene diphosphonate (Tc-MDP) in the metaphysis and shaft of the rat femur as affected by hypophysectomy and hormonal replacement with growth hormone and thyroxine. Two hours following injection of Tc-MDP, the metaphysis and a specimen of shaft were obtained and the metaphysis-to-shaft radioactivity ratio was measured. By five days after hypophysectomy the metaphysis-to-shaft ratio fell from a control value of 3.8 ± 0.2 (mean ± s.e.) to 2.4 ± 0.2 ($p < 0.05$) and remained significantly decreased throughout the 30-day study. When daily hormonal replacement with 0.5 mg of bovine growth hormone and 10 μgm of thyroxine (both administered intraperitoneally) was given, beginning on the eighth day after hypophysectomy, the metaphysis-to-shaft ratio of Tc-MDP returned to control levels in twelve days. This model demonstrates the effect of growth hormone and thyroxine on the distribution of Tc-MDP, and may be useful as a radiobioassay of net circulating skeletal growth-promoting activity.

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Skeletal growth is dependent primarily on growth hormone and thyroid-stimulating hormone from the anterior pituitary (*1*). Both act indirectly, growth hormone through stimulation of hepatic synthesis and release of one or more somatomedins (*2*), and thyroid-stimulating hormone through stimulation of thyroidal synthesis and release of thyroxine. In addition, a number of other factors also influence growth, including other hormones, genetic endowment, nutrient environment, and ionic concentrations (*1*). While methods such as radioimmunoassay are available to measure many of these individual factors, no simple quantitative method is currently available to measure the net effect of the various growth-promoting and -inhibiting factors on the metabolism of bone growth centers.

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We have developed a method for quantification of metaphyseal clearance of Tc-99m methylene diphosphonate (Tc-MDP), and in hypophysectomized animals we have demonstrated a significant decrease in metaphyseal clearance and a return to normal following hormonal replacement with growth hormone and thyroxine.

METHODS AND MATERIALS

Technique. Two hours following intravenous injection of Tc-MDP, the rat was killed and both femurs were dissected free (Fig. 1A). The distal ephiphysis was then mechanically separated from the shaft (Fig. 1B) and the remainder of the femur was cut with a fine saw one-eighth inch (~3 mm) proximal to the midline groove of the ephiphyseal disc and at the distal and proximal ends of the lateral femoral protuberance (Fig. 1C). The weight and counts per minute were determined for epi-

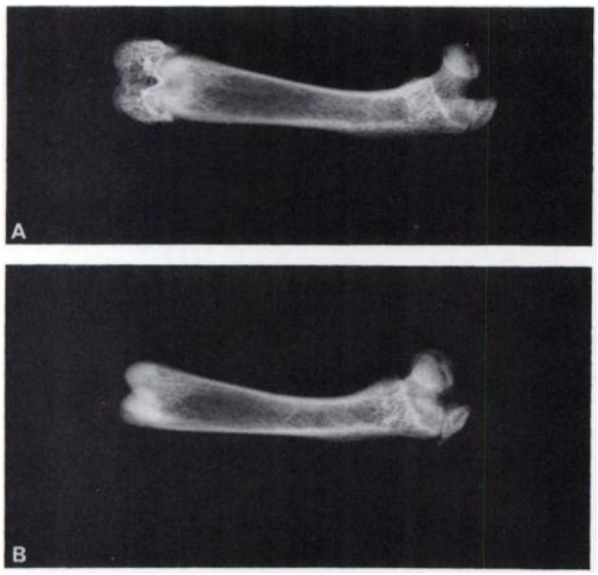

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Fig. 1. (A) Radiograph of rat femur demonstrates distal cartilaginous epiphyseal disc. (B) Same, following mechanical removal of epiphysis. (C) Diagram of specimens obtained from rat femur by mechanically dissociating epiphysis, then transecting rest of bone with a fine saw ~3 mm proximal to midline groove of the epiphyseal disc, and at both ends of lateral femoral protuberance.

physeal (E), metaphyseal (M), distal shaft (DS), and proximal shaft (PS) specimens (Fig. 1C).

Histology. Dissected femoral ephiphyseal and metaphyseal specimens from both femurs of two control and two hypophysectomized rats were fixed for 24 hr in neutral 1% gluteraldehyde in phosphate-buffered saline, decalcified in 0.1 ethylenediamine tetra-acetic acid, pH 6.9, until soft, then washed with phosphate-buffered saline and embedded in methacrylate (*3*). Two-micron sections were stained with 0.15% toluidine blue, pH 6.5, rinsed with distilled water, dehydrated with acetone, cleared in xylol, and mounted in Permount*.

Animal protocol. All animals were 9-wk-old male Sprague-Dawley rats weighing approximately 200 g at the time of arrival. Each data point represents the average of four to eight animals. Control rats were killed at 2, 8, 15, 20, and 30 days after arrival. Hypophysectomized rats were obtained two days after hypophysectomy and received supplemental feedings of water with 5% dextrose for the first 5 days after arrival. These rats were killed at 2, 5, 8, 15, 20, and 30 days after hypophysectomy.

To determine the effect of hormonal replacement, hypophysectomized rats were given intraperitoneal in-

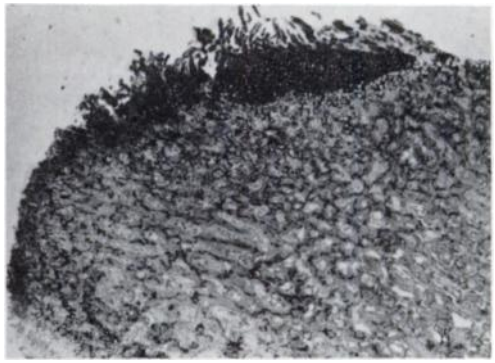

Fig. 2. Longitudinal section through metaphyseal specimen demonstrates cartilage from epiphyseal disc along distal end of the metaphyseal specimen, and entire histologic metaphysis within metaphyseal specimen.

jections of 0.5 mg of bovine growth hormone and 5 μgm of thyroxine daily, beginning on the eighth day following hypophysectomy. Animals were killed following 7, 12, and 22 days of treatment.

Data analysis. Relative metaphyseal uptake of Tc-MDP was analyzed by a number of methods including: (a) M cpm/g ÷ X cpm/g, where X equals each of the other specimens individually, and (b) M cpm/g ÷ (E + M + DS + PS) cpm/g (Fig. 1C). Each method uses another part of the same femur to normalize uptake for injected radioactivity.

RESULTS

Histology. Sections through the metaphyseal and epiphyseal specimens from seven of eight femurs demonstrated that the cleavage plane lay within the cartilage of the epiphyseal disc, and therefore that the entire metaphysis lay within the metaphyseal specimen (Fig. 2). In the case of the one femur in which the separation of epiphysis from metaphysis was not through the cartilaginous disc, we suspected at the time of separation that the cleavage plane was atypical because of its rough appearance.

Data analysis. The measurement with the smallest relative standard deviation and most significant separation between hypophysectomized rats and controls was: M cpm/g ÷ DS cpm/g; only this measurement is reported in this paper. However, the ratio E cpm/g ÷ DS cpm/g is potentially useful in monitoring for separations between epiphyseal and metaphyseal specimens in which part of the histologic metaphysis separates with epiphyseal specimen. In this case the ratio E cpm/g ÷ DS cpm/g will be unusually high.

Effect of hypophysectomy and hormonal replacement. The results of metaphyseal uptake in control, hypophysectomized, and hormonally replaced rats are shown in Fig. 3. There were no significant differences in metaphyseal uptake in control rats between 9 and 13 wk of age. By five days following hypophysectomy, the meta-

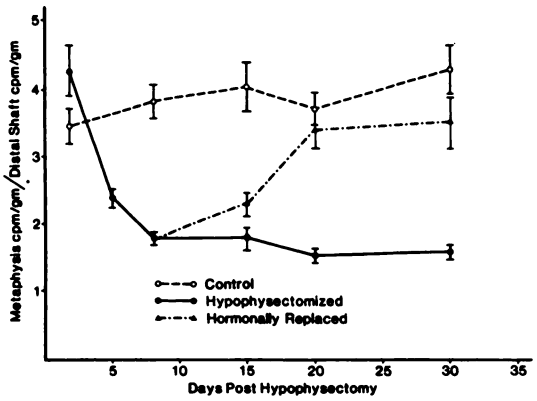

Fig. 3. Metaphysis-to-shaft ratio of Tc-MDP (mean ± s.e.m.) for control, hypophysectomized, and hormonally replaced rats as a function of time after hypophysectomy. There is no significant change in metaphysis-to-shaft ratio in controls over the 30-day period. By five days after hypophysectomy there is significant decrease in metaphyseal clearance, which persists through 30 days. When hypophysectomized rats are treated with growth hormone and thyroxine, metaphysis-to-shaft ratio returns to near normal levels in 12 days.

physis-to-shaft ratio fell from an age-matched control value of 3.8 ± 0.2 (mean ± s.e.) to 2.4 ± 0.2 ($p < 0.05$). From 8 to 30 days, the uptake remained relatively constant.

The hypophysectomized animals that were treated with growth hormone and thyroxine, starting at eight days after hypophysectomy, showed an increase in the metaphysis-to-shaft ratio after seven, twelve, and twenty-two days of treatment; the values at twelve and twenty-two days were significantly ($p < 0.01$) higher than in untreated animals and not significantly different from control values.

DISCUSSION

We have developed a method for quantitating the relative metaphyseal clearance of Tc-MDP in the rat femur. The finding that this measurement decreases following hypophysectomy and returns to normal following replacement with growth hormone and thyroxine suggests that it might be useful in measuring net circulating skeletal growth-promoting activity. Previous methods, such as the measurement of tibial length, also measure the net circulating skeletal growth-promoting activity (*4*), but do so in terms of change in physical dimensions rather than change in metabolic activity.

The net circulating skeletal growth-promoting activity can also be determined in an entirely in vitro system by measuring the incorporation of radioactive sulfur into cartilage obtained from hypophysectomized animals (*5,6*). However, the in vitro approach is more tedious than the method described here, is less physiologic in that the target tissue is in vitro rather than in vivo during the time of stimulation, and does not allow chronic stimulation of the target tissue. Also Tc-MDP uptake is directly related to bone formation in the present model.

In a previous in vivo study using a radiotracer, the uptake of strontium-85 in the hock joint of chicks during the first six weeks of life was measured with an external probe detector (*6*). When the measurements were corrected for hock-joint weight, the uptake decreased with time as growth velocity declined (*6*). However, the effect of altering circulating growth factors on strontium-85 uptake was not determined. In a semiquantitative approach in patients with active and inactive acromegaly, a moderately strong correlation was shown between visual evaluation of Tc-MDP images and the serum growth-hormone level (*9*).

Currently measurement of individual factors that influence skeletal growth is not a suitable alternative to measuring the net circulating skeletal growth-promoting activity. A number of skeletal growth-promoting factors have been identified and there is evidence for several growth-inhibiting factors (*2*). Short stature in pygmies is associated with normal levels of growth hormone and insulin-like growth factor-II, but a significantly decreased level of insulin-like growth factor-I (*8*). On the other hand, poor growth in children with chronic renal failure is associated with high levels of somatomedin-A suggesting the presence of increased levels of inhibiting factors (*10*).

Increase in skeletal length is a result of chondrocyte multiplication and matrix synthesis in the epiphyseal disc, followed by removal of epiphyseal disc cartilage and its replacement by bone in the metaphysis (*11*). Although the exact mechanism of clearance of Tc-MDP by bone is not known (*12*), the increased band of Tc-MDP seen in images of growing bones has been shown to correspond to the metaphysis by autoradiographic studies (*13*). An ideal radiobioassay of metaphyseal clearance would separate the metaphysis completely from the epiphyseal cartilage disc and epiphysis distally, and from the diaphysis proximally. This is difficult to accomplish by transection of the femur because the metaphysis cannot be identified visually and because it does not form a flat plane (Fig. 1). Our method of initial mechanical dissociation of the epiphysis leaves only a thin layer of cartilage on the distal side of the metaphysis (Fig. 2). However, the limits of the metaphysis are not as well defined on its diaphyseal side, so a larger amount of bone ($\frac{1}{8}$ inch) is left on the proximal side (Fig. 1C).

We have applied a similar, but noninvasive, strategy in two groups of children deficient in growth hormone (*14*). In each patient one tenth of the usual weight-adjusted dose of Tc-MDP was injected, and two hours later a single ten-minute anterior digital image of the knees and lower two thirds of the femurs was stored. This image was used to calculate the ratios for femoral metaphysis to shaft and femoral metaphysis to injected

dose, before and after four months of growth-hormone therapy. In the first group of children there was no change in the metaphysis-to-shaft ratio but there was a significant increase in the metaphysis-to-dose ratio (*14*). The evidence suggested that in these children growth hormone caused an equal increase in uptake of Tc-MDP in the metaphysis and shaft. In a second group of seven children, all seven demonstrated an increase in both the metaphysis-to-dose and metaphysis-to-shaft ratios (unpublished data). We cannot explain the difference in results between the two groups although a more accurate, automatic, edge-detection algorithm was used to analyze the images in the second group. In addition, unlike the hypophysectomized rats, neither patient group contained individuals who were thyroxine-deficient.

We conclude that in the rat the femoral metaphysis-to-shaft ratio of clearance of Tc-MDP is decreased by hypophysectomy and restored by hormonal replacement with growth hormone and thyroxine. This measurement may be useful as a radiobioassay for net circulating skeletal growth-promoting activity.

FOOTNOTE

* Fisher Scientific, Fairlawn, New Jersey.

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