In vivo Effect of Parathyroid Hormone and/or Calcitonin on the Lysosomal Enzyme Activity of Rat Bone

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Synopsis

The effect of parathyroid hormone (PTH) and/or calcitonin in vivo on the lysosomal enzyme activity of rat bone was studied. PTH caused a significant increase in the acid-phosphatase and β-glucuronidase activity of the rat tibia in a dose-related manner. Calcitonin also caused a significant increase in the acid-phosphatase of the tibia. Administration of calcitonin did not counteract the PTH-induced increase in the enzyme activity. These results may give rise to some doubt on the hypothesis that lysosomal enzymes play a vital role in the process of bone resorption.

Recently the role of lysosomal enzymes in the process of bone resorption has attracted widespread interest. Vaes, (1968) has demonstrated in vitro that the release of lysosomal enzymes from the bone into the medium was markedly increased by the addition of parathyroid hormone (PTH). Based on these data, they have proposed a hypothesis that lysosomal enzymes in the bone may play a vital role in the PTH-induced bone resorption (Vaes, 1968). Calcitonin, on the other hand, has been shown to inhibit PTH induced bone resorption (Hirsch and Munson, 1969). However, its mechanism of action is still obscure. We have therefore studied whether or not PTH stimulates lysosomal enzymes of the bone also in vivo and whether calcitonin antagonizes its effect.

Materials and Methods

Experimental procedures

Female Wistar rats of 50 gm. b.w. of the uniform age were maintained on Oriental rat chow and then fasted overnight before use. All the rats were surgically thyroparathyroidectomized 24 hr before the experiments. Three types of experiments were conducted:

I. Effect of PTH on the enzyme activity of the rat tibia

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Twenty thyroparathyroidectomized rats, randomly divided into 4 equal groups, were s.c. injected with 100 USP units of PTH and 0, 30, 120, 480 MRC mU of porcine calcitonin respectively at the same time. Left tibiae were taken at 6 hr after the injection.

Analytical Procedure

Acid phosphatase, alkaline phosphatase and β-glucuronidase activity was measured and expressed according to the method of Vaes and Jacques (1965). One per cent homogenate of the tibia was prepared by homogenizing frozen tibia in ice cold 0.25 M sucrose with bone crusher and Waring blender after the removal of the bone marrow. The homogenate was then mixed with 0.1% Triton X-100, centrifuged at 3000 rpm 3 min and the supernatant was used for the enzyme assay. Protein was determined by Lowry’s method (1951).

Hormone Preparations

Porcine calcitonin (13 MRC U/mg), a gift from Armour pharmaceutical Co. was dissolved in 16% gelatin before use. Parathyroid Injection from Eli Lilly and Co. was used as PTH.

Statistical Analysis

The data in each experiment were subjected to analysis of variance. The standard errors were obtained from the residual error term of the analysis of variance. The means were compared using the multiple comparison test of Hartley (1967).

Results

1. PTH stimulation of the enzyme activity of the tibia in thyroparathyroidectomized rat.

a) Time course study

Administration of 200 USP unit of PTH caused a significant increase in the activity of acid phosphatase and β-glucuronidase of the tibia 6 hr after the injection. The enzyme activities at 6 hr after PTH injection were significantly higher than those at 0 time with P<0.05. Increase in the alkaline phosphatase activity of the tibia was also noted 6 hr following the injection of PTH. (Fig. 1.)

b) Effect of graded doses

As shown in Figure 2, PTH stimulated the activity of β-glucuronidase and acid phosphatase of the tibia of thyroparathyroidectomized rat in a dose related manner. The increase in the alkaline phosphatase activity after PTH was not dose dependent.

Fig. 1. PTH stimulation of the enzyme activity of the tibia in thyroparathyroidectomized rat. In this and succeeding figures, each point represents the mean of 5 rats. Vertical bar indicates standard error.

2. Effect of calcitonin on the enzyme activity of the tibia

a) Time course study

A significant increase in the activity of acid and alkaline phosphatase of the tibia was noted 6 hr after the administration of 100 MRC mU of calcitonin. However, calcitonin did not cause a significant change in the β-glucuronidase activity of the tibia. (Fig. 3).

b) Effect of graded doses

As shown in Figure 4, calcitonin increased the acid and alkaline phosphatase activity of the tibia in a dose related manner.

3. Effect of simultaneous administration of PTH and calcitonin on the enzyme activity of the rat tibia.

In the previous experiments (Fig. 1 and 3) both PTH and calcitonin increased the acid phosphatase activity of the tibia when administered separately. Simultaneous administration of 100 U of PTH and graded dose of calcitonin (30–480 MRC mU) further increased the enzyme activity in a dose related manner. (Fig. 5) Similar dose related increase in the β-glucuronidase and alkaline phos-
Fig. 2. Dose related increase in the enzyme activity of the rat tibia after 12.5~200 U of PTH. Enzyme activity was measured 6 hr after the injection.

Fig. 3. Calcitonin stimulation of acid and alkaline phosphatase activity of the tibia in thyroparathyroidectomized rat.

Discussion

In 1968, Vaes has presented some evidence which suggests that lysosomal enzymes in the bone are directly involved in the process of PTH induced bone resorption. Using tissue culture technique, he has shown that the release of lysosomal enzymes from the calvariae into the medium was markedly increased by the addition of PTH. In view of the facts that some of these enzymes are particularly abundant in bone cells (Changus, 1957; Burstone, 1960) and may degrade mucopolysaccharides and proteins (De Duve, 1959), the constituents of the organic matrix of bone, it is possible that PTH causes bone resorption through the increase in these enzymes. Calcitonin has been shown to inhibit bone resorption and antagonize the effect of PTH. Heersch (1969) has demonstrated in vitro that calcitonin significantly inhibited the increased release of lysosomal enzymes in the PTH stimulated bone cultures. Since Vaes (1968) has demonstrated the increase in the enzyme activities of the
Fig. 4. Dose related increase in the acid and alkaline phosphatase activity of the rat tibia after 25~100 mU of calcitonin. Enzyme activity was measured 6 hr after the injection.

Fig. 5. Failure of calcitonin in antagonizing PTH induced increase in the acid phosphatase activity of the rat tibia. Enzyme activity was measured 6 hr after the s.c. injection of 100 U of PTH and 30~480 MRC mU of porcine calcitonin given simultaneously at the different site.

Our finding that PTH stimulates the activity of lysosomal enzymes of the rat tibia is consistent with Vaes' observation (1968), giving further support to the concept that lysosomal enzymes play a vital role in the PTH induced bone resorption. Since we have measured the enzyme activities of the bone homogenate treated with Triton X, our results could not be compared with those of Vaes' who have measured the activities of the released enzymes from the bone into the medium. However, the fact that calcitonin also stimulates the lysosomal enzyme activity of the rat tibia without antagonizing the PTH effect is contradictory to this hypothesis. In our experiment, 30 mU of calcitonin caused a significant increase in acid-phosphatase activity of the tibia in thyroparathyroidectomized rat. However, the same dose of calcitonin did not increase the enzyme activity of the bone when the rats were simultaneously treated with 100 USP units of PTH. Although it is difficult to find a reasonable explanation for this, one possibility is that the site of calcitonin action is already saturated with PTH. Reynolds (1968) failed to show any correlation between
demineralization and the accumulation of lysosomal enzymes. Using tissue culture system, Raisz et al. (1968) have demonstrated a marked inhibition of the removal of calcium from the bone by calcitonin. However, the release of previously incorporated hydroxyproline was not blocked by the addition of calcitonin. Similar results were obtained by Reynolds (1968) who showed that calcitonin could completely inhibit vitamin A stimulated removal of calcium from rat calvaria in tissue culture, but did not inhibit the vitamin A induced release of proteolytic enzymes into the medium. These data may suggest that calcitonin primarily blocked calcium removal and had no direct effect on the process of matrix resorption. In view of the fact that lysosomal enzymes may also play some role in the process of new bone formation as suggested by Mclean (1968), it might be possible that calcitonin stimulation of lysosomal enzymes in the bone may reflect the increased new bone formation due to calcitonin. However, this possibility needs further evaluation and at present it is still difficult to explain why calcitonin stimulates lysosomal enzyme activity in vivo.

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References


