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Effects of Thyroparathyroidectomy and Thyrocalcitonin on the Bone Acid Phosphatase Activity and the Serum Calcium Concentration in Rats treated with Lead Acetate

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The effect of thyroparathyroidectomy and thyrocalcitonin on the bone acid phosphatase activity and the serum calcium level in lead-treated rats was studied. Lead administration tc intact rats produced a significant increase in bone acid phosphatase activity and serum calcium level. Thyroparathyroidectomy significantly decreased the bone acid phosphatase activity and the serum calcium level in intact normal rats. However, lead administration prevented this reduction. Thyrocalcitonin injection significantly depressed the bone acid phosphatase activity and the serum calcium level elevated by the administration of lead to thyroparathyroidectomized rats and intact normal rats. The present results suggest that the increase in the bone acid phosphatase activity and the serum calcium level caused by lead administration is independent upon the action by parathyroid hormone.

Parathyroid hormone causes bone resorption by stimulating the release of lysosomal acid hydrolases from the bone cells and by creating the acidic conditions for the action of these enzymes,[2)] and is able to increase the level of calcium in serum.[3)] On the other hand, the hypercalcemic effect of lead is obliterated by the injection of thyrocalcitonin and actinomycin D which inhibit the action of bone resorption by parathyroid hormone.[4)] Recently, we found that the lysosomal acid hydrolases activity in bone cells of rats was increased by the administration of lead.[5)] From these facts, hypercalcemic effect by lead seemed to be largely related to the action of parathyroid hormone. The present study was therefore undertaken to evaluate whether these effects of lead is dependent upon the presence of parathyroid hormone. This report describes the effects of thyroparathyroidectomy and thyrocalcitonin on the acid phosphatase activity, a lysosomal enzyme, in bone cells and the calcium concentration in serum of rats treated with lead.

Materials and Methods

Male Wistar strain rats, each weighing approximately 120 g, were utilized in experiment. They were kept at a room temperature of $25 \pm 1°$ and fed purina chow and tap water *ad libitum*. Lead acetate was dissolved in distilled water to a concentration of 20 mg lead/ml. All injections of this solution (1.0 ml/100 g body weight) were given as single intraperitoneal administration to rats. All surgical procedures were done under light ether anesthesia. The thyroparathyroid gland complex was removed with fine forceps. One hour later the animals were administered lead and after a subsequent 48 hr were killed. Thyrocalcitonin (lyophilized porcine thyrocalcitonin, 68 MRC U/mg protein, Armour Pharmaceutical Company) of 0.4 MRC U/0.5 ml, diluted in distilled water, was injected subcutaneously at 47 hr after lead administration. The animals were killed 1 hr after thyrocalcitonin injection.

Metaphyseal bone fragment from the femur were harvested and cleaned of soft tissue and marrow in saline solution at 0°. The pooled bone fragments were finely minced with scissors and then were immersed

1) Location: *2-2-1, Oshika, Shizuoka.*

2) G. Vaes, "Lysosomes in Biology and Pathology," Vol. I, ed. by J.T. Dingle and H.B. Fell, Inc., North-Holland Publishing Company, Amsterdam, 1969, pp. 217—253.
3) R.V. Talmage and J.R. Elliot, *Endocrinology* **62**, 717 (1958).
4) M. Yamaguchi and T. Yamamoto, *Toxicol. Appl. Pharmacol.* **29**, 223 (1974).
5) M. Yamaguchi, H. Sato, and T. Yamamoto, *Chem. Pham. Bull.* (Tokyo), **23**, 1789 (1975).

in 3 ml of 7.5 mM barbital buffer (pH 7.4) at 0°, and disrupted for 30 sec with an ultrasonic device.[6] The supernatant centrifugated at 6000 *g* for 5 min were used for the enzyme assays and total protein determination. Acid phosphatase activity was determined by the method of Linhardt and Walter,[7] Messer, *et al.*[6] based on the release of *p*-nitrophenol from *p*-nitrophenylphosphate. Acid phosphatase was assayed in a total volume of 1.2 ml in the presence of 5.5 mM *p*-nitrophenylphosphate and 50 mM citrate buffer, pH 5.0. The enzyme tests were carried out at 37° for 30 min. The reaction was stopped by the addition of 4.0 ml of 0.1N NaOH. Appropriate blanks were always run in parallel to the tests and substracted from the observed values. Enzyme activity was calculated as μmoles of *p*-nitrophenol liberated per min per mg protein. Total protein was determined by the method of Lowry, *et al.*[8]

Blood samples were centrifuged immediately after the collection, and serum was separated. The calcium in serum was determined by atomic absorption spectrophotometry (Perkin-Elmer, Model 303) after precipitation of the protein with 10% trichloroacetic acid.[9]

Results

Lead was administered to normal and thyroparathyroidectomized rats and bone acid phosphatase activity and serum calcium level were measured (Table I). Thyroparathyroidectomy significantly decreased the bone acid phosphatase activity in normal rats. However, lead administration prevented this reduction. In the animals administered lead after thyroparathyroidectomy, thyrocalcitonin injection inhibited significantly an increase in the bone acid phosphatase activity induced by lead administration. The treatment of thyroparathyroidectomized rats with thyrocalcitonin did not significantly change the bone acid phosphatase activity when compared with that of the untreated rats. On the other hand, lead administration caused a significant increase in serum calcium concentration of intact normal and thyroparathyroidectomized rats. Thyrocalcitonin injection significantly reduced the serum calcium concentration elevated by lead administration to thyroparathyroidectomized rats.

TABLE I. Effect of Thyroparathyroidectomy and Thyrocalcitonin on Bone Acid Phosphatase Activity and Serum Calcium Concentration of Rats treated with Lead

Treatment	Number of rats	Bone acid phosphatase activity[a]	Serum calcium (mg/100 ml)
Normal	7	0.185±0.009[b]	10.6±0.44
Lead	6	0.263±0.010[c]	14.1±0.56[c]
Thyroparathyroidectomy			
Control	7	0.136±0.008[c]	5.1±0.72
Lead[d]	8	0.227±0.015[e]	9.2±0.53[e]
Lead + thyrocalcitonin[f]	7	0.155±0.011[g]	6.5±0.24[g]
Thyrocalcitonin	6	0.110±0.007	4.3±0.17

a) Enzyme activity was expressed as μmoles *p*-nitrophenol liberated /min/mg protein.
b) mean±SEM for 6 or 8 animals
c) differs from respective normal mean, $p < 0.01$ (Student's *t* test)
d) Doses of 20 mg Pb/100 g was intraperitoneally given 1 hr after thyroparathyroidectomy, and 48 hrs later the rats were killed.
e) differs from respective control of thyroparathyroidectomy, $p < 0.01$
f) The animals were subcutaneously given 0.4 MRC U/100 g of thyrocalcitonin at 47 hrs after the administration of lead. The animals were killed 1 hr after the injection of thyrocalcitonin.
g) differs from respective lead of thyroparathyroidectomy, $p < 0.01$

The experiments shown in Fig. 1 and Fig. 2 was attempted to examine the correlation between the bone acid phosphatase activity and the serum calcium concentration increased by lead administration. The time course of the increase of the bone acid phosphatase and

6) H.H. Messer, W.D. Armstrong, and L. Singer, *Proc. Soc. Expt. Biol. Med.* **143**, 690 (1973).
7) K. Linhardt and K. Walter, "Methods of Enzymatic Analysis," ed., by H.V. Bergmeyer, Academic Press, New York, 1965, p. 783.
8) O.H. Lowry, N.J. Rosebrough, A.L. Farr, and R.J. Randall, *J. Biol. Chem.* **193**, 265 (1951).
9) J.B. Willis, *Nature* **16**, 249 (1960).

the serum calcium after lead administration is shown in Fig. 1. The bone acid phosphatase activity exhibited a rapid elevation 4 hr after lead administration when compared with that of control animals received the distilled water. The serum calcium concentration remained normal for 4 hr and then increased significantly at 12 hr after lead administration. The effect of thyrocalcitonin on the serum calcium concentration and bone acid phosphatase activity of intact normal and lead-treated rats is shown in Fig. 2. Thyrocalcitonin injection, at the dose used, exhibited a rapid, marked fall in the bone acid phosphatase activity of the lead-treated but not of normal rats, probably because of the higher initial enzyme activity in the lead-treated rats. Just after the injection of thyrocalcitonin to the lead-treated rats, the greatest fall of the serum calcium concentration paralleled the most striking decrease of acid phosphatase activity.

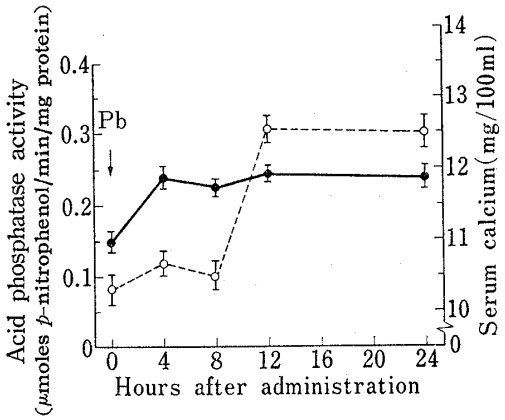

Fig. 1. Effect of Lead on the Bone Acid Phosphatase Activity and the Serum Calcium Concentration of Rats

The animals were intraperitoneally given 20 mg Pb/100 g body weight. Each point represents the mean value of 5 or 6 animals. The vertical lines give the SEM.
●—● : bone acid phosphatase, ○—○ :serum calcium

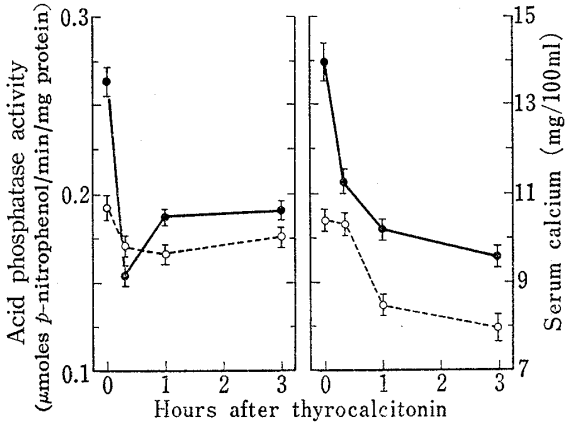

Fig. 2. Effect of Thyrocalcitonin on the Bone Acid Phosphatase Activity and the Serum Calcium of Rats treated with Lead

The animals were subcutaneously given 0.4 MRC U/100 g of thyrocalcitonin at 47 hrs after the administration of lead. The animals were killed 20 min, 1 hr and 3 hrs after the injection of thyrocalcitonin. Each point represents the mean value of 5 or 6 rats. The vertical lines give the SEM.
○···○ : control, ●—● : lead

Discussion

It is well known that acid phosphatase activity, a lysosomal enzyme in bone cells[2] and calcium concentration in serum[3] is increased in response to parathyroid hormone. Acid phosphatase has been used as an indicator of bone resorption.[2] On the other hand, lead administration significantly increased the bone acid phosphatase activity[5] and serum calcium concentration.[4] It was possible, therefore, that these effects of lead might be due largely to an activation of the release or action of parathyroid hormone.[5] But this is unlikely for the present findings that lead inhibited the fall of the bone acid phosphatase activity and of the serum dalcium concentration following surgical thyroparathyroidectomy. The most probable mechanism for the effect of lead would be that lead may directly induce an increase of the acid phosphatase activity of bone cells to culminate in the increase of serum calcium level.

The present experiments showed a good correlation between the increase in the acid phosphatase activity in bone cells and the calcium concentration in serum of the lead-treated rats. The increase of bone acid phosphatase activity induced by lead administration occurred earlier than the increase of serum calcium concentration. By the injection of thyrocalcitonin, the greatest fall in the serum calcium concentration of the lead-treated rats occurred simultaneously with the decrease in bone acid phosphatase activity. Presumably, the exhibition of hypercalcemic effect by lead may be caused by the bone resorption through the increase in lysosomal acid hydrolases in bone cells induced by lead administration.