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The Increase of Acid Hydrolases Activity in Bone Tissue of Rats Treated with Lead Acetate

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The effect of a single intraperitoneal administration of lead acetate on acid hydrolases activity in bone tissues of rat was studied. The administration of lead to rats produced a persistent increase in acid phosphatase activity, while alkaline phosphatase activity decreased. There was a significant increase in β-glucuronidase, β-N-acetylglucosaminidase, and β-galactosidase activities in bone tissues as early as 1 day after lead administration. These enzymes activity, at three dose levels (5, 10, and 20 mg lead per 100 g body weight), significantly increased when compared with the values obtained from rats received control injection with distilled water. In the present study, it is demonstrated that the administration of lead could cause an increase in lysosomal acid hydrolases activity in the bone tissues of rat.

Introduction

We recently reported that the serum calcium concentration was increased by the administration of lead to rats and that thyrocalcitonin and actinomycin D inhibited the hypercalcemic effect by lead.[2] The antagonism of thyrocalcitonin and actinomycin D to parathyroid hormone appears to be due to their inhibitory effect on the differentiation of osteocytes to osteoclasts which is induced by parathyroid hormone.[3] Accordingly, our result suggest that the hypercalcemic effect of lead may be largely dependent upon the presence of parathyroid hormone.

On the other hand, the release of acid hydrolases from lysosome in the bone cells is in good correlation with the progress of bone resorption, and the activity of these enzymes is increased in response to parathyroid hormone.[4] The present study was therefore undertaken to investigate the effect of lead on acid hydrolases activity in soluble fraction extracted from bone tissue of rats. We found that the administration of lead is significantly increased the acid hydrolase activity in bone of rats.

Materials and Methods

Experimental Animals——Male Wistar strain rats, each weighing approximately 120 g, were utilized in these experiments. They were kept at a room temperature of 25±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.

Tissue Preparation——The animals were bled by cardiac puncture. Metaphyseal bone fragments obtained from the femur were carefully dissected out, cleaned free of any adhering muscular or connective tissue and is steeped in saline solution at 0°. The marrow cells were removed to histological observe little cells. The pooled metaphyseal bones were finely minced with scissors and then immersed in 3 ml of 7.5 mM barbital buffer (pH 7.4) at 0°. The bone tissues were disrupted for 30 sec with an ultrasonic device.[5] The supernatant centrifugated at 6000 *g* for 5 min were used for measurement of the total enzyme activities.

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Enzyme Assays and Analytical Procedures——Acid and alkaline phosphatase activities were determined by the method of Lindhardt and Walter,[6] Messer, *et al.*,[5] based on the release of *p*-nitrophenol from *p*-nitrophenylphosphate. Inorganic phosphate liberated from β-glycerophosphate and phenylphosphate was determined by the method of Allen.[7] The enzyme assay techniques for β-glucuronidase, β-N-acetylglucosaminidase, and β-galactosidase were based on those used for the calvaria by Vaes and Jacques.[8] The enzyme tests were carried out at 37°. Acetate or citrate buffers were generally used: they were prepared by mixing in various proportions equimolar solutions of acetic acid or citric acid and sodium acetate or sodium citrate. Appropriate blanks, in which the enzyme was added to the incubation flasks just after the enzymic reaction was stopped, were always run in parallel with the tests to subtract from the observed values. The enzymic activities were expressed as mμmoles of the decomposed substrate per min per mg of protein. Protein was determined by the method of Lowry, *et al.*[9] with bovine serum albumin as a standard.

Results

The time course of the increase in acid and alkaline *p*-nitrophenylphosphatase activities in bone tissues of rat after a single intraperitoneal administration of lead is shown in Fig. 1. The animals were bled at various time intervals after lead administration. Acid *p*-nitrophenylphosphatase activities significantly ($p<0.01$) increased at 1 day after lead administration when compared with that of control animals, and this enzyme activity was still elevated even after 6 days. An entirely different pattern was observed for alkaline *p*-nitrophenylphosphatase. This enzyme activity decreased rapidly, reached the minimal level at 2 days after lead administration, and returned to control levels. Using *p*-nitrophenylphosphate, β-glycerophosphate, and phenylphosphate as the substrate, the bone acid phosphatase activity examined 3 days after lead administration was found to be significantly ($p<0.01$) increased (Table I). It should be noted that acid phenylphosphatase activity in bone tissues of the lead-treated rats increased approximately 6 times of control values. Thus, the administration of lead caused a significant increase in the activity of acid phosphatase, a lysosomal enzyme.

Then, the lead administration was examined for the effect on β-glucuronidase, β-N-acetylglucosaminidase, and β-galactosidase which have been shown to be associated with lysosomes in bone cells of rats. The time course of the increase of these enzymes activities in bone tissues of rats after a single intraperitoneal administration of lead is shown in Fig. 2. As early as 1 day after lead administration there was a significant ($p<0.01$) increase of all the enzyme activities. The bone acid hydrolases activities measured after 3 days were summarized in Table II.

The effects of varying dose of lead on β-glucuronidase, β-N-acetylglucosaminidase, and β-galactosidase in bone tissues of rat was examined 1 day after the administration (Fig. 3). The activity of these acid hydrolases significantly ($p<0.01$) increased at all the doses tested when compared with that of control animals receiving the injection of distilled water alone. In a dose of 10 mg lead per 100 g body weight, β-glucuronidase activity increased approximately 1.5 times, β-galactosidase activity virtually doubled and β-N-acetylglucosaminidase activity was elevated to 3 times of the control.

Discussion

The present study demonstrate clearly that the administration of lead could increase the activity of lysosomal acid hydrolases of bone tissues of rat. Non-lysosomal and lysosomal enzymes, alkaline phosphatase and acid hydrolases, showed an entirely different pattern

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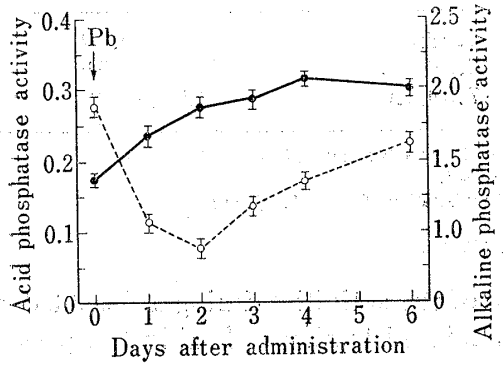

Fig. 1. Effect of a Single Intraperitoneal Administration of Lead (20 mg/100 g) on Acid and Alkaline *p*-Nitrophenylphosphatase Activities in Bone Tissues of Rats

Acid *p*-nitrophenylphosphatase 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 reaction was stopped by the addition of 4.0 ml of 0.1N NaOH. Alkaline *p*-nitrophenylphosphatase was measured in a total volume of 1.1 ml in the presence of 5.5 mM *p*-nitrophenylphosphate and 50 mM glycine buffer, pH 9.9. The reaction was stopped by the addition of 10.0 ml of 0.02N NaOH. Enzyme activities were expressed as μmoles *p*-nitrophenol liberated/min/mg protein. The intraperitoneal administration of a control distilled water lacking lead produced no significant effect on the bone phosphatase activities at any of the time intervals between 1 and 3 days. Each point represents the mean value of 5 or 6 animals. The vertical lines give the SEM. ●—●, acid *p*-nitrophenylphosphatase; ○—○, alkaline *p*-nitrophenylphosohatase.

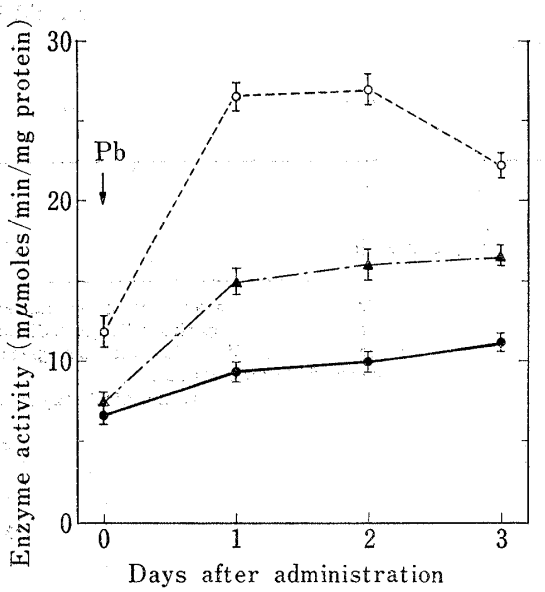

Fig. 2. The Time Course of the Increase of β-Glucuronidase, β-N-Acetylglucosaminidase, and β-Galactosidase Activities in Bone Tissues of Rats after a Single Intraperitoneal Administration of Lead

β-Glucuronidase activity was measured in a total volume of 1 ml of 0.1M acetate buffer, pH 5.0, with 1.5 mM-phenolphtalein glucuronidate as substrate. The reaction was stopped by the addition of 3.0 ml of a solution containing glycine (0.133M), NaCl (0.067M) and Na_2CO_3 (0.083M), pH 10.7. β-N-Acetylglucosaminidase was determined in a total volume of 1 ml of 0.1M citrate buffer, pH 5.0, with 8 mM *o*-nitrophenyl N-acetyl-β-D-glucosaminide as substrate. The reaction was stopped by the addition of 1.5 ml of 2.75% (w/v) trichloroacetic acid. β-Galactosidase was assayed in a total volume of 1.0 ml of 0.1M-citrate buffer, pH 3.6, with 3.75 mM *o*-nitrophenyl β-D-galactoside as substrate. The reaction was stopped by the addition of 1.5 ml of 2.75% trichloroacetic acid. The intraperitoneal administration of a control distilled water lacking lead produced no significant effect on the acid hydrolases activity at any of the time intervals between 1 and 3 days. Each point represents the mean value of 5 or 6 animals. The vertical lines give the SEM.
●—●: β-glucuronidase
○···○: β-N-acetylgluco-saminidase
▲—·—▲: β-galactosidase

TABLE I. Effect of the Various Substrate on Acid Phosphatase Activity in Bone Tissue of Rats administered Lead

Substrate	Number of rats	Enzyme activity[a]	
		Control[b]	Lead[c]
p-Nitrophenylphosphate	8	189.1±8.0[d]	272.1±13.2[e]
β-Glycerophosphate	6	13.3±2.1	21.3± 1.1[e]
Phenylphosphate	6	64.0±4.3	398.0±18.0[e]

a) Enzyme activity was expressed as mμmoles of the decomposition of substrate/min/mg protein.
b) Animals were intraperitoneally given distilled water lacking lead.
c) Animals were killed 3 days after a single intraperitoneal administration of 20 mg lead/100 g.
d) mean±SEM for 6 or 8 animals
e) Significantly greater than control enzyme activity: $p<0.01$ (Student's *t* test).

Table II. Effect of Lead on Acid Hydrolases Activityin Bone Tissue of Rats

Enzyme	Number of rats	Enzyme activity[a]	
		Control[b]	Lead[c]
β-Glucuronidase	6	6.9±0.6[b]	11.1±0.5[e]
β-N-Acetylglucosaminidase	6	12.3±1.3	22.3±0.5[e]
β-Galactosidase	6	7.5±1.0	16.1±1.0[e]

a) Enzyme activity was expressed as mμmoles of the decomposition of substrate/min/mg protein.
b) Animals were intraperitoneally given distilled water lacking lead.
c) Animals were killed 3 days after a single intraperitoneal administration of 20 mg lead/100 g.
d) mean±SEM for 6 animals
e) Significantly greater than control enzyme activity: $p<0.01$ (Student's t test).

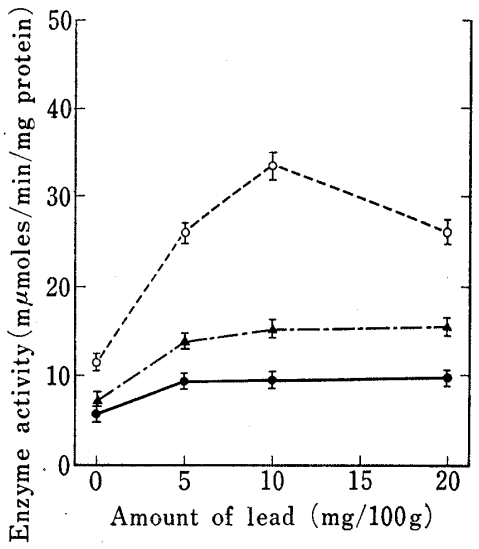

Fig. 3. Effect of a Single Intraperitoneal Administration of the increased Amount of Lead on β-Glucuronidase, β-N-acetylglucosaminidase, and β-Galactosidase in Bone Tissues of Rats

Animals were killed 1 day after lead administration. Control animals received the injection of distilled water. Each point represents the mean acid hydrolases activity of 5 or 6 animals. The vertical lines give the SEM.
●—●, β-glucuronidase;
○·····○, β-N-acetylglucosaminidase
▲—·—▲, β-galactosidase

of the change of the activities during at least 3 days following the administration of lead in the present experiments.

Previously, we reported that thyrocalcitonin[10-12] and actinomycin D[13] inhibiting the bone resorption induced by parathyroid hormone obliterated the hypercalcemic effect of lead.[2] From this fact, the hypercalcemic effect of lead seemed to be largely dependent upon the presence of parathyroid hormone, which could cause bone resorption by stimulating the release of lysosomal acid hydrolases from the bone cells[4] and by creating the acidic conditions for the action of these enzymes.[4,14] The present results show clearly that the administration of lead caused a persistent increase in the activity of lysosomal acid hydrolases, suggesting the hyperfunction of parathyroid gland to result in the stimulated bone resorption. Indeed, a great amount of lead is accumulated in bone compacta as a result of lead administration,[15] and that lead mobilizes the calcium in bone[16] and bone calcium is markedly lowered.[15] In order to clarify the point, histological parameter, such as number of osteoclasts or relative area of resorbing surface, would be adopted as more reliable indicators of bone resorption rate. As the cellular mechanisms, moreover, acid production enabling the released lysosomal enzymes to function for bone resorption remains to be determined.

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