Influence of Calcitonin on Bone Phosphatases and Phosphate Release in Organ Culture (37392)

H. H. MESSER, W. D. ARMSTRONG, AND L. SINGER

Department of Biochemistry (Health Sciences), University of Minnesota, Minneapolis, Minnesota 55455

A major action of calcitonin on bone is the inhibition of bone resorption (1, 2), although it may also stimulate bone formation (3). Because of the presumed association of acid and alkaline phosphatases with these processes, the influence of calcitonin on the two enzymes was investigated. Acid phosphatase, a lysosomal enzyme, has been used as an indicator of bone resorption, and the activity of this enzyme is increased in response to parathyroid hormone (4). Alkaline phosphatase, a nonlysosomal enzyme, has been implicated in bone formation, although its precise role remains obscure (5). We have investigated the effects of calcitonin and parathyroid hormone on both enzymes, and on the release of phosphate from bones maintained in organ culture.

Methods. Acid and alkaline phosphatase activities were determined in half-calvaria taken from 5-day-old mice, either freshly dissected or after 24 or 48 hr of culture. A stationary culture system similar to that described by Reynolds and Dingle (6) was used with half-calvaria cultured in individual dishes containing 1 ml medium. The control culture medium consisted of 95% medium CMRL 1066 plus 5% heat-inactivated horse serum.[1] Penicillin (50 U/ml) and streptomycin (50 μg/ml) were added as antibiotics. When calcitonin was included in the medium, a preparation of highly purified salmon ultimobranchial calcitonin[2] was dissolved in medium CMRL 1066 and added to the culture medium at a final concentration of 4.0 mU/ml. An additional 4.0 mU/ml was added after 24 hr of culture. A further group of half-calvaria was cultured in medium containing 0.5 U parathyroid hormone/ml.[3] Cultures were maintained at 37.5° for 24 or 48 hr, in an atmosphere of 95% air, 5% carbon dioxide.

Enzyme assays were performed on bone extracts, obtained by ultrasonic disruption of the bone cells. Half-calvaria were immersed in 2 ml 7.5 m*M* barbital buffer (pH 7.4) at 0°, and disrupted for 15 sec with an ultrasonic device.[4] Aliquots of the supernatant were used for the enzyme assays and total protein determinations. Acid and alkaline phosphatase activities were determined by the method of Lindhardt and Walter (7), based on the release of *p*-nitrophenol from *p*-nitrophenyl phosphate. Acid phosphatase was assayed at pH 4.8 (0.05 *M* citrate buffer) and alkaline phosphatase at pH 9.5 (0.1 *M* bicarbonate–carbonate buffer plus 5.0 m*M* magnesium chloride). Total protein was determined by the method of Lowry *et al.* (8). Enzyme activities were calculated as micromoles of *p*-nitrophenol liberated per minute per milligram of protein. Acid phosphatase activity in the culture medium was too low for accurate determination, a difficulty encountered by other workers (4). Accordingly, enzyme assays were confined to bone extracts.

The release of calcium and phosphorus under identical culture conditions was also

[1] Culture medium, serum and antibiotic solutions were obtained from Grand Island Biological Co., Grand Island, NY.

[2] Salmon calcitonin, Lot UBC 20, kindly provided by Dr. D. H. Copp, Physiology Department, University of British Columbia, Vancouver 8, British Columbia.

[3] Parathyroid extract, Eli Lilly and Co., Indianapolis, IN.

[4] Biosonik III, Bronwill Scientific Co., Rochester, NY.

TABLE I. Alkaline and Acid Phosphatase Activities of Half-calvaria from 5-Day-Old Mice Before and After Culture in the Presence of Calcitonin or Parathyroid Hormone.

	Enzyme activity[a]			Change (%) in 48 hr
	0 hr	24 hr	48 hr	
Acid phosphatase				
Control	0.076 ± 0.002[b]	0.069 ± 0.003	0.078 ± 0.004	+3.1
0.5 U/ml PTH		0.078 ± 0.007	0.097 ± 0.008[e]	+28.1
4.0 mU/ml CT		0.092 ± 0.006[d]	0.113 ± 0.014[e]	+50.0
Alkaline phosphatase				
Control	1.30 ± 0.07	1.25 ± 0.06	1.18 ± 0.10	−8.5
0.5 U/ml PTH		1.34 ± 0.10	1.20 ± 0.09	−7.5
4.0 mU/ml CT		1.29 ± 0.05	1.53 ± 0.05[c]	+18.2

[a] Expressed as μmoles *p*-nitrophenol liberated/min/mg protein.
[b] Mean ± SEM for 6 or 8 experiments.
[c] Significantly greater than initial (0 hr) enzyme activity: $p < 0.05$; [d] $p < 0.02$; [e] $p < 0.01$.

determined, for a 48 hr culture period only. The culture media were analyzed before and after culture for calcium (9) and phosphorus (10), and the bones were ashed and analyzed after culture for calcium and phosphorus. The release of calcium and phosphorus was calculated as a percentage of that originally present in each bone.

Results and Discussion. The enzyme activities prior to culture and after 24 and 48 hr of culture are recorded in Table I. Bones cultured in control medium (without added parathyroid hormone or calcitonin) maintained approximately constant acid phosphatase activity during 48 hr of culture, and there was a small but not significant decline in alkaline phosphatase activity. Parathyroid hormone promoted a significant ($p < 0.01$) increase in acid phosphatase activity after 48 hr, relative to initial (0 hr) enzyme activity. At the same time there was a slight decrease in alkaline phosphatase activity. Both alkaline and acid phosphatase activities of calvaria exposed to 4.0 mU calcitonin/ml in the culture medium were significantly elevated above control values after 48 hr of culture. The increase in acid phosphatase activity (50.0% greater than initial activity, p value <0.01) was greater than the increase in alkaline phosphatase activity (18.2%, p value <0.05). The increase in acid phosphatase activity in response to calcitonin occurred more rapidly than in response to parathyroid hormone, but was not significantly greater after 48 hr.

Parathyroid hormone stimulated an increased release of calcium and phosphorus from the bones, relative to control values (Table II). The release of phosphorus exceeded that of calcium, on a percentage basis. Calcitonin promoted an uptake of calcium by bone from the culture medium and inhibited the loss of phosphorus from the bones to the medium. However, phosphorus was released from the bones at the same time as calcium was taken up by the bones.

TABLE II. Percentage Release in 48 hr of Calcium and Phosphorus from Bones Exposed to Calcitonin or Parathyroid Hormone.

	Calcium	Phosphorus	Significance level[a]
Control	11.2 ± 0.8[b]	13.6 ± 0.8	ns
0.5 U/ml PTH	16.8 ± 1.2	22.2 ± 1.4	$p < 0.02$
4.0 mU/ml CT	−2.0 ± 0.4	5.2 ± 0.2	$p < 0.001$

[a] Comparing calcium release with phosphorus release by Students' *t* test.
[b] Mean ± SEM for 5 or 6 experiments in all cases.

The increase in alkaline phosphatase activity produced by calcitonin is consistent with the concept of a stimulus to bone formation, since alkaline phosphatase activity tends to be high when bone formation is rapid (5). However, the elevated acid phosphatase activity is contrary to what might be predicted, since increased acid phosphatase activity has been thought to be associated with an increased rate of bone resorption (4). The interrelationship between acid and alkaline phosphatase in bone is complex and the activity of both enzymes may be elevated in cases of rapid bone turnover as in growing animals and in Paget's disease (11). In this study, the bone cell types synthesizing these enzymes were not identified, and the normal substrates *in vivo* are not known. Thus it is difficult to assign precise roles to these enzymes in bone formation and bone resorption.

Vaes (12) reported that calcitonin inhibited the release of 2 lysosomal enzymes, β-glucuronidase and N-acetyl-β-glucosaminidase from bones exposed to both parathyroid hormone and calcitonin in culture. However, Reynolds (13) did not observe any inhibition by calcitonin of vitamin A-stimulated lysosomal enzyme release. Thus the question of the effect of calcitonin on lysosomal enzymes has not been fully resolved, and in the present study the increased acid phosphatase activity in the bones may have resulted from an inhibition of the release of lysosomal enzymes from the bone cells.

Talmage (14) proposed that calcitonin is primarily a phosphate-regulating hormone and should stimulate "an enzyme concerned with phosphate release from organic components." Such a role for acid phosphatase (or alkaline phosphatase) could explain the release of phosphorus from bones at the same time as calcium was being taken up.

Summary. Calcitonin promoted an increase in both alkaline and acid phosphatase activities of half-calvaria from 5-day-old mice when the bones were cultured for 48 hr. Calcitonin inhibited the release of phosphorus from bones, relative to the release from control and parathyroid hormone-treated bones, and promoted an uptake of calcium from the culture medium. The results support the concept of a role for calcitonin in phosphate metabolism.

This study was supported by Grant No. DE-01850, National Institute for Dental Research, Bethesda, MD.

1. Friedman, J., and Raisz, L. G., Science **150**, 1465 (1965).

2. Copp, D. H., Annu. Rev. Physiol. **32**, 61 (1970).

3. Gaillard, P. J., and Thesingh, C. W., "Proceedings of Symposium on Thyrocalcitonin and the C-Cells," p. 238. Heinemann, London (1968).

4. Vaes, G., J. Cell Biol. **39**, 676 (1968).

5. Bourne, G. H., *in* "The Biochemistry and Physiology of Bone" (G. H. Bourne, ed.), 2nd ed., Vol. 2, p. 79. Academic Press, New York (1972).

6. Reynolds, J. J., and Dingle, J. T., Calcif. Tissue Res. **4**, 339 (1970).

7. Linhardt, K., and Walter, K., *in* "Methods of Enzymatic Analysis" (H. V. Bergmeyer, ed.), p. 783. Academic Press, New York (1965).

8. Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J., J. Biol. Chem. **193**, 265 (1951).

9. Kepner, B. L., and Hercules, D. M., Anal. Chem. **35**, 1238 (1963).

10. Fiske, C. H., and SubbaRow, Y., J. Biol. Chem. **66**, 375 (1925).

11. Dixon, T. F., and Perkins, H. R., *in* "The Biochemistry and Physiology of Bone" (G. H. Bourne, ed.), p. 287. Academic Press, New York (1956).

12. Vaes, G. S., J. Dent. Res. **51**, 362 (1972).

13. Reynolds, J. J., Calcif. Tissue Res. **4**, (Suppl.) 52 (1970).

14. Talmage, R. V., Clin. Orth. **67**, 211 (1969).

Received March 5, 1973. P.S.E.B.M., 1973, Vol. 143.