

RAPID ACTIVATION OF THE MEDULLARY BONE OSTEOCLAST CELL SURFACE BY PARATHYROID HORMONE

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ABSTRACT

Quantitative transmission electron microscope methods were used to determine the response of functionally inactive avian medullary bone osteoclasts to parathyroid hormone (PTH). Egg-laying Japanese quail were used during a period of the egg cycle when medullary bone was not being resorbed for egg shell calcification and when medullary bone osteoclasts were functionally inactive. Ruffled borders adjacent to bone surfaces were rarely, if ever, found on these cells. 20 min after the administration of PTH, over 70% of the osteoclast profiles had ruffled borders adjacent to bone surfaces. These ruffled borders were bounded by filamentous-rich "clear zones" and resembled ruffled borders found on functionally active cells. There was also a marked increase in plasma calcium levels after PTH administration. This study demonstrates that PTH stimulates the *de novo* generation of ruffled borders on osteoclasts *in vivo* and suggests that osteoclasts may be involved in the acute regulation of calcium metabolism by exogenous PTH.

KEY WORDS bone · osteoclasts ·
parathyroid hormone · cell surface

The role of osteoclasts in resorption and remodeling of structural bone of the skeleton is well established, but much remains to be learned about the role of these cells in the regulation of calcium metabolism. Parathyroid hormone (PTH) controls body fluid calcium levels principally by regulating the removal of calcium from bone. After PTH administration, marked elevations in plasma calcium levels occur within several hours in most mammals (13), and in minutes in egg-laying birds (3). PTH increases osteoclast populations *in vivo* (14), stimulates osteoclast activity *in vitro* (5), and accelerates the rate of skeletal remodeling (4). However, these effects are usually noted long after changes in plasma calcium levels, leading to

the belief that osteoclasts are not rapidly responsive to PTH and thus not involved in acute regulation of calcium metabolism.

Assessment of the actual response of osteoclasts to PTH had been difficult because, in most types of bone, osteoclasts are a nonuniform population of cells in heterogeneous states of activity. Recently, we described an *in vivo* system in which osteoclasts are functionally and predictably synchronized (9). During the egg-laying cycle in birds, osteoclasts resorb medullary bone deposits to supplement dietary calcium for egg shell formation (10). In quail medullary bone, osteoclasts appear to be active only during the period of egg shell calcification and apparently become inactive during the remainder of the cycle (9).

The purpose of this study is to examine changes in osteoclast structure and function after PTH

TABLE I
Effect of 20-min Parathyroid Hormone Treatment on Plasma Calcium Levels and Osteoclast Morphology

	Plasma calcium levels			Osteoclasts with ruffled borders
	0 min	20 min	net change	
	<i>mg % ± SD</i>			<i>% ± SD</i>
Control	25.4 ± 0.8	25.5 ± 0.7	+0.1 ± 0.9	<1
PTH	24.1 ± 1.1	28.7 ± 1.5	+4.6 ± 1.6*	77 ± 3*

* Significantly greater than controls, $P < 0.005$.

administration to quail in vivo. This report demonstrates that the cell surface of functionally inactive osteoclasts is rapidly activated after PTH administration.

MATERIALS AND METHODS

Egg-laying Japanese quail (*Coturnix Coturnix Japonica*) were studied during the phase of the egg cycle 4 h after ovulation. At this time, the ovum is in the oviduct but has not reached the shell gland, no medullary bone resorption is occurring (10), and osteoclasts lack the ruffled borders adjacent to bone surfaces characteristic of actively resorbing cells (9).

Intraperitoneal injections of bovine parathyroid hormone (Parathyroid injection, Eli Lilly & Co., Indianapolis, Ind.) were given at a dose of 100 IU/kg body weight to five hens, while five hens received 0.9% sodium chloride and served as controls. Blood samples for the determination of plasma calcium levels (2) were taken by cardiac puncture at the time of PTH or saline administration and 20 min later from the same birds. At this latter time, fragments of medullary bone were quickly taken from the femora and immersed in fixative. The tissues were fixed in 0.1 M phosphate-buffered osmium tetroxide or in 0.1 M sodium cacodylate-buffered 3% glutaraldehyde. Some of the bone fragments fixed in glutaraldehyde were decalcified in 4% EDTA, and all glutaraldehyde-fixed tissues were subsequently fixed in 0.1 M sodium cacodylate-buffered osmium tetroxide. Tissues were embedded in Epon, and thin sections cut with diamond knives were mounted on 100-mesh Formvar-coated grids and examined on a JEOL 100B or 100S electron microscope.

The numbers of osteoclasts with ruffled borders were estimated in the electron microscope in the following manner. Only one thin section from each tissue fragment was used. Unobstructed complete profiles of osteoclasts which were adjacent to a bone surface and contained at least one nucleus were counted. The presence or absence of a ruffled border was determined on each osteoclast examined. 200 different osteoclasts were examined from each bird.

The data are expressed as the mean ± the standard deviation (±SD). The differences in the data were tested for significance by analysis of variance (1).

RESULTS

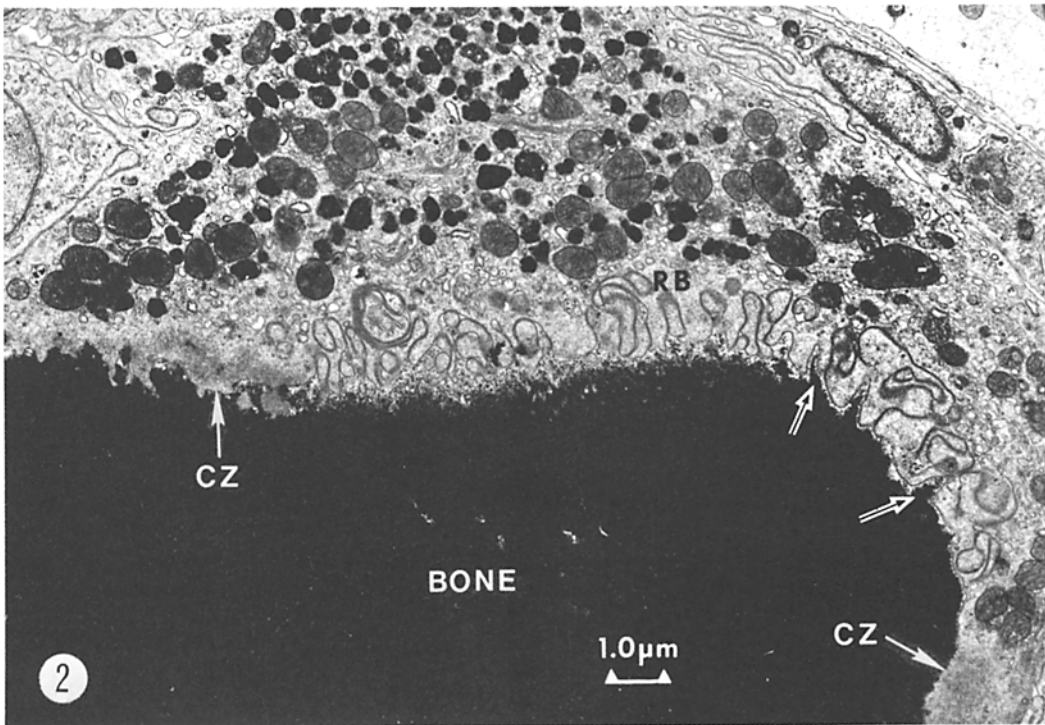
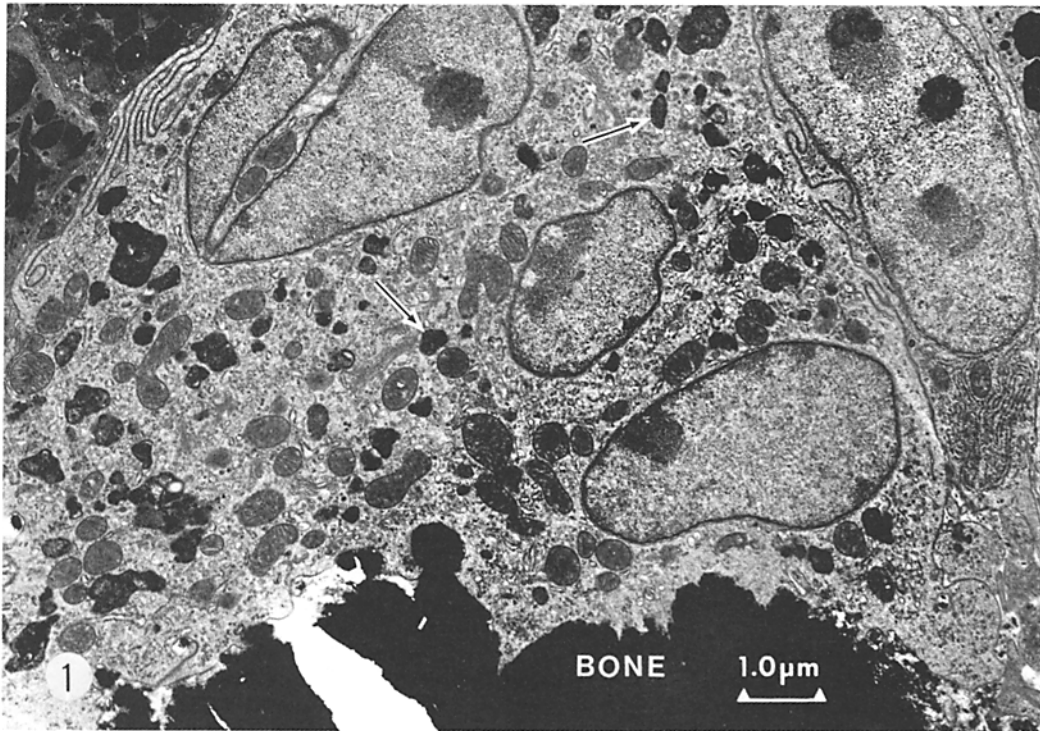
20 min after PTH administration, there is a marked increase in plasma calcium levels (Table I). No significant change in plasma calcium occurred in the control hens during the experimental period.

Four h after ovulation, medullary bone osteoclasts are round in shape and largely removed from bone surfaces except for a small filamentous zone that remains attached to the bone surface (9). Ruffled borders, channel expansions, and associated vacuoles are not found on these cells (Table I, Fig. 1). These observations suggest that osteoclastic bone resorption is not occurring at this time in the egg-laying cycle (9).

Dramatic changes in osteoclast cell surface structure are observed in the birds treated for 20 min with PTH. The majority of osteoclast profiles now have ruffled borders adjacent to bone sur-

FIGURE 1 Electron micrograph of a medullary bone osteoclast taken at 4 h after ovulation. At this time in the egg-laying cycle, calcium is not being removed from medullary bone for egg shell formation. Ruffled borders on osteoclasts are rarely, if ever, found at this time. Numerous electron-dense bodies (arrows) are present in the cytoplasm of these cells. Osmium tetroxide fixation, × 9,000.

FIGURE 2 Low-power electron micrograph of a medullary bone osteoclast taken at 4 h after ovulation and 20 min after administration of parathyroid hormone. Osteoclasts are extended along bone surfaces, and ruffled borders (RB) bounded by filamentous "clear zones" (CZ) are present. Small mineral crystals and larger pieces of mineralized tissue can be found between the folds of the ruffled border (arrows). Osmium tetroxide fixation. × 6,500.



faces (Table I, Fig. 2). The ruffled borders are characterized by numerous invaginations and fingerlike processes. Small mineral crystals can be found between the membrane folds of the ruffled border (Fig. 2) and in small vacuole-like structures associated with the ruffled border. The ruffled border region of these cells is bounded by the filamentous ectoplasmic region termed the clear zone (reference 12; CZ, Fig. 2).

DISCUSSION

This study demonstrates that exogenous PTH causes a very rapid change in the cell surface structure of the medullary bone osteoclast in egg-laying Japanese quail. Osteoclasts which were functionally inactive develop ruffled borders adjacent to bone surfaces within 20 min after PTH administration. Plasma calcium levels markedly increase during the same time. These observations support the concept that PTH-induced increases in plasma calcium levels in birds is due, at least in part, to mobilization of calcium from bone (11).

The rapid development of ruffled borders on osteoclasts after PTH administration, as demonstrated in this study, supports the findings obtained in other species that PTH causes an increase in the frequency of ruffled borders on osteoclasts in vitro (5) and an increase in the area occupied by ruffled borders on osteoclasts in vivo (6). It should be noted that the administration of the hypocalcemic hormone, calcitonin, results in the rapid disappearance of ruffled borders on osteoclasts in vitro (5, 7) and in vivo (8). These findings suggest that the osteoclast may play an important role in effecting rapid changes in blood calcium levels.

Avian medullary bone appears to offer unusual opportunity for examination of bone cell function. The cells are functionally synchronized by natural physiological mechanisms and are rapidly responsive to exogenous stimuli, as demonstrated in this study with PTH. Medullary bone has no apparent structural function, as the trabeculae are anisotropic. Because medullary bone cells are probably not involved in or influenced by the processes associated with skeletal remodeling for the maintenance of structural bone, they are well suited for the study of action of hormones, vitamins, and other factors on bone cell structure and function in relation to mineral metabolism.

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