pH dependence of bone resorption: mouse calvarial osteoclasts are activated by acidosis

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Meghji, Sajeda, Matthew S. Morrison, Brian Henderson, and Timothy R. Arnett. pH dependence of bone resorption: mouse calvarial osteoclasts are activated by acidosis. Am J Physiol Endocrinol Metab 280: E112–E119, 2001.—We examined the effects of HCO3− and CO2 acidosis on osteoclast-mediated Ca2+ release from 3-day cultures of neonatal mouse calvaria. Ca2+ release was minimal above pH 7.2 in control cultures but was stimulated strongly by the addition of small amounts of H+ to culture medium (HCO3− acidosis). For example, addition of 4 meq/l H+ reduced pH from 7.12 to 7.03 and increased Ca2+ release 3.8-fold. The largest stimulatory effects (8- to 11-fold), observed with 15–16 meq/l added H+, were comparable to the maximal Ca2+ release elicited by 1,25-dihydroxyvitamin D3 [1,25(OH)2D3; 10 nM], parathyroid hormone (10 nM), or prostaglandin E2 (1 μM; the action of these osteolytic agents was attenuated strongly when ambient pH was increased from ~7.1 to ~7.3). CO2 acidosis was a less effective stimulator of Ca2+ release than HCO3− acidosis over a similar pH range. Ca2+ release stimulated by HCO3− acidosis was almost completely blocked by salmon calcitonin (20 ng/ml), implying osteoclast involvement. In whole mount preparations of control half-calvaria, ~400 inactive osteoclast-like multinucleate cells were present; in calvaria exposed to HCO3− acidosis and to the other osteolytic agents studied, extensive osteoclastic resorption, with perforation of bones, was visible. HCO3− acidosis, however, reduced numbers of osteoclast-like cells by ~50%, whereas 1,25(OH)2D3 treatment caused increases of ~75%. The results suggest that HCO3− acidosis stimulates resorption by activating mature osteoclasts already present in calvarial bones, rather than by inducing formation of new osteoclasts, and provide further support for the critical role of acid-base balance in controlling osteoclast function.

THE SKELETON CONTAINS a massive reserve of base that is available as a “fail-safe” mechanism to buffer protons if the kidney and lungs are unable to maintain acid-base balance within narrow physiological limits (8, 15). The deleterious effects of systemic acidosis on the skeleton have been recognized at least since the early part of this century (5, 18). More recent in vivo studies have suggested that the bone loss associated with acidosis is not due to passive physicochemical processes but involves enhanced osteoclastic resorption (9, 22).

Experiments with disaggregated rat osteoclasts cultured on cortical bone or dentine wafers provided the first direct evidence for the stimulatory action of extracellular protons on cell-mediated bone resorption. Resorption pit formation by rat osteoclasts in culture media buffered nonphysiologically, with the use of HEPES only, was activated by progressive acidification from pH 7.4 to 6.8 (3). Cultured osteoclasts are also stimulated to resorb in physiologically buffered media when pH is reduced either by decreasing HCO3− concentration or by increasing PCO2 (2). Recent work has shown that rat osteoclasts are particularly sensitive to extracellular pH in the range 7.2–7.0, such that shifts of <0.1 unit are sufficient to cause changes of several-fold in pit formation (6).

Experiments with cultured mouse calvaria showed that calcium efflux from bones was stimulated much more strongly when extracellular pH was reduced by decreasing HCO3− concentration (metabolic acidosis) than by increasing PCO2 (respiratory acidosis) (11, 12). The aims of the present study were 1) to investigate the effects of small extracellular pH changes resulting from HCO3− and CO2 acidosis on osteoclastic resorption in cultured calvaria; 2) to compare the effects of acidosis on calvarial resorption with those of “classical” osteolytic agents and to determine whether the action of these agents was dependent on ambient acidification; and 3) to investigate whether acidosis stimulates resorption by activating mature osteoclasts already present in calvarial bones or by inducing formation of new osteoclasts.

MATERIALS AND METHODS

Reagents. 1,25-Dihydroxyvitamin D3 [1,25(OH)2D3] was kindly provided by Dr K. W. Colston (St. George’s Hospital Medical School, London, UK). BWA 70C, a 5-lipoxygenase inhibitor of the iron ligand class, containing the hydroxamic acid release; carbon dioxide; bicarbonate ion; acid-base balance in controlling osteoclast function.

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acid chelating group, was a gift of the Wellcome Foundation (Beckenham, Kent, UK). MK 886, a selective inhibitor of the 5-lipoxygenase-activating protein (FLAP), was donated by Merck Frosst (Kirkland, QC, Canada). Other reagents, unless specified, were purchased from Sigma (Poole, Dorset, UK).

*Mouse calvarial bone resorption assay.* The method, which measures bone resorption as $\text{Ca}^{2+}$ release from neonatal mouse calvaria, was similar to that described in detail by Meghji et al. (24). Briefly, 5-day-old MF1 mice were killed by cervical dislocation. The frontoparietal bones were removed and trimmed of any adhering connective tissue and interparietal bone, with care being taken not to damage the periosteum. Dissected calvaria were pooled, washed free of blood and adherent brain tissue in Hanks’ balanced salt solution, and then divided along the sagittal suture. Half-calvaria were cultured individually on 1-cm$^2$ stainless steel grids (Minimesh, FDP quality, Expanded Metal, West Hartlepool, UK) in 6-well plates with 1.5 ml of BGJ medium, 5% heat-inactivated fetal calf serum, 100 U/ml penicillin, and 100 µg/ml streptomycin (ICN Biomedicals, Basingstoke, Harls, UK) at the air-liquid interface in a humified CO$_2$ incubator. After an initial 24-h preincubation period, the medium was removed and replaced with control or test media. Prostaglandin E$_2$ (PGE$_2$), 1.25% (OH)$_3$D$_3$, indomethacin, BWA 70C, and MK 886 were dissolved in ethanol vehicle for use; the final concentration of ethanol in cultures did not exceed 1:500.

Each experimental group consisted of five individual cultures. Included in each experiment were groups of half-calvaria that had been killed by three cycles of freeze-thawing with liquid nitrogen. The cultures were then incubated for 72 h without further medium changes and without the incubator door being opened, so as to ensure constant CO$_2$ levels and minimize pH fluctuations. Culture medium acidification was achieved either by adding small amounts of concentrated HCl to culture medium as described by Murrills et al. (31), resulting in decreased HCO$_3$ concentration (metabolic acidosis) or by increasing incubator CO$_2$ tension (respiratory acidosis).

After 72 h, experiments were terminated by withdrawal of culture medium and washing of bones once with PBS, followed by fixation in 95% ethanol-5% glacial acetic acid for 10 min. Incubator Pco$_2$ was determined by immediate measurement of a culture medium sample by use of a blood gas analyzer (Radiometer ABL 330, Copenhagen, Denmark). The mean final pH of each treatment group was determined by removal and pooling of a 100-µl sample from each replicate; the pooled samples were then reequilibrated with CO$_2$ in the incubator before measurement with the blood gas analyzer. Slight differences in CO$_2$ tension between groups were normalized to the initially measured value using pH-Pco$_2$ calibration curves constructed for BGJ medium, as previously described (31). Bicarbonate concentrations were calculated by the Radiometer ABL 330 blood gas analyzer by use of the Henderson-Hasselbalch equation.

Calcium concentrations in culture medium at the end of experiments were measured colorimetrically by autoanalyzer (Chem Lab Instruments, Essex, UK) with the following procedure. Samples were acidified with excess 1 M HCl and subjected to continuous flow dialysis against the metal complexing agent creosolphthalein complexone (CPC) to separate $\text{Ca}^{2+}$ from proteins; 8-hydroxyquinoline (2.5 g/l) was added to samples to eliminate Mg$^{2+}$ interference. Dialyzed Ca$^{2+}$ bound to CPC was then determined after reaction with 2-amino-2-methylpropano-1-ol; the absorbance of the resultant purple-colored solution was measured at 570 nm. The basal calcium concentration of the BGJ medium after addition of 5% heat-inactivated fetal calf serum was 2.00 mM. All measurements were performed blind on coded samples.

Whole mount histology. After fixation/decalcification with 95% ethanol-5% glacial acetic acid, calvaria were stained for tartrate-resistant acid phosphatase (TRAP) (20, 23) by means of a Sigma kit 387-A and were mounted in melted glycerol jelly. The numbers of TRAP-positive multinucleated osteoclasts (two or more nuclei) were assessed blind on coded samples by means of transmitted light microscopy.

Statistics. Statistical comparisons, where appropriate, were made by one-way analysis of variance, with the use of Bonferroni’s correction for multiple comparisons. Representative data are presented as means ± SE for 5 replicates. Results are shown for representative experiments that were each repeated at least three times.

**RESULTS**

**Effect of HCO$_3^-$ acidosis on calvarial resorption.** Basal levels of $\text{Ca}^{2+}$ release in nonacidified control bones (pH 7.20–7.25) were very low after 3 days of culture. Acidification of culture medium by addition of small amounts of H$^+$ as HCl resulted in a steep increase in $\text{Ca}^{2+}$ release from live bones. For example, decreasing the pH of the culture medium from 7.21 (control) to 7.17 by addition of 3 meq/l H$^+$ reduced HCO$_3^-$ concentration from 13.2 to 12.2 mmol/l, resulting in a 2.8-fold increase in $\text{Ca}^{2+}$ release, and decreasing the pH to 6.94 by addition of 15 meq/l H$^+$ reduced HCO$_3^-$ concentration to 7.1 mmol/l, causing an 8.3-fold stimulation of $\text{Ca}^{2+}$ release. In contrast, in bones killed by freeze-thawing, there was a marked influx of $\text{Ca}^{2+}$, evidenced by a decrease in culture medium $\text{Ca}^{2+}$ concentration; the magnitude of this influx was slightly reduced with progressive acidification (Fig. 1).

**Effect of CO$_2$ acidosis.** When culture medium was acidified from 7.12 to 6.90 by increasing incubator Pco$_2$ from 54.5 to 87.2 mmHg, only a small, nonsignificant increase in $\text{Ca}^{2+}$ release from calvaria was observed after 3 days of culture. The stimulation of $\text{Ca}^{2+}$ release resulting from CO$_2$ acidosis was markedly lower than that elicited by HCO$_3^-$ acidosis at comparable pH values. However, further acidification to pH 6.81 by increasing Pco$_2$ to 108.5 mmHg resulted in a 5.3-fold stimulation of $\text{Ca}^{2+}$ release compared with control. This stimulation occurred in the face of an increase in HCO$_3^-$ concentration from 15.8 to 22.5 mM (Fig. 2).

**Effects of inhibitors.** HCO$_3^-$ acidosis-stimulated $\text{Ca}^{2+}$ release (resulting from addition of 15 meq/l H$^+$) was completely blocked by salmon calcitonin (20 ng/ml; Fig. 3). Thus $\text{Ca}^{2+}$ release stimulated by HCO$_3^-$ acidosis was due to osteoclastic resorption. $\text{Ca}^{2+}$ release resulting from severe CO$_2$ acidosis was also blocked by salmon calcitonin (20 ng/ml; data not shown). HCO$_3^-$ acidosis-stimulated $\text{Ca}^{2+}$ release was additionally completely blocked by the cyclooxygenase inhibitors indomethacin (Fig. 4) and ibuprofen (data not shown) and was inhibited by the 5-lipoxygenase inhibitors, BWA 70C (Fig. 5A) and MK 886 (Fig. 5B), suggesting that the effect may be mediated by both prostaglandins and leukotrienes.

Effects of “classical” resorption stimulators. The maximal effects of the osteolytic agents 1,25(OH)$_2$D$_3$
(10 nM), bovine parathyroid hormone-(1–34) (PTH, 20 ng/ml) and PGE₂ (1 μM) on Ca²⁺ release from 3-day calvarial cultures were similar and were equivalent in magnitude to the effect of HCO₃⁻ acidosis at pH 6.94 (15 meq/l added H⁺; Fig. 6). In most experiments, PTH, 1,25(OH)₂D₃, and PGE₂ treatments themselves resulted in slight acidification of culture medium.

In an additional series of experiments, we examined the dependence of the osteolytic action of PGE₂, 1,25(OH)₂D₃, and PTH on ambient acidification in 3-day cultures of mouse half-calvaria. Addition of 15 meq/l OH⁻ as NaOH increased final medium pH from ~7.1 in control cultures to ~7.3, resulting in marked attenuation of Ca²⁺ release in all treatment groups (Fig. 7). Addition of equivalent amounts of Na⁺ as NaCl to culture medium was without effect on bone resorption. In the case of PGE₂-stimulated bones, increasing pH from 7.12 to 7.33 reduced resorption by 80%.

**Histology (TRAP staining) and cell counting.** In whole mount preparations of half-calvaria cultured in control medium for 3 days and then stained to demonstrate tartrate-resistant acid phosphatase, ~200–400 inactive TRAP-positive osteoclast-like multinucleate cells were typically present; resorption cavities visualized by the TRAP staining were small and relatively inactive (Figs. 8A and 9). The appearance of freshly isolated bones (i.e., not cultured) was similar. In calvaria exposed to HCO₃⁻ acidosis (~12 meq/l H⁺), extensive osteoclastic resorption cavities, with characteristic scalloped edges, were evident (Fig. 8B); resorption was sometimes sufficiently aggressive to cause complete perforation of bones. In bones exposed to severe CO₂ acidosis (108.5 mmHg) at pH 6.82, osteoclasts in scalloped resorption bays were also clearly evident (Fig. 8C). Extensive resorption cavities were also ob-

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**Fig. 1.** Stimulatory effect of small decreases in medium pH, achieved by adding H⁺ (i.e., HCO₃⁻ acidosis) as HCl, on Ca²⁺ release from live mouse half-calvaria cultured for 3 days (open bars). Addition of 0, 3, 6, 9, 12, and 15 meq/l H⁺ to culture medium resulted in calculated HCO₃⁻ concentrations of 13.2, 12.2, 10.9, 10.4, 8.5, and 7.1 mmol/l, respectively after 3 days of culture. In dead bones killed by freeze-thawing (hatched bars), a net Ca²⁺ influx occurred that was slightly reduced as pH decreased (~). Pco₂ was 36.3 mmHg. Values are means ± SE (n = 5). Significantly different from nonacidified control: *P < 0.05; **P < 0.01; ***P < 0.001.

**Fig. 2.** Stimulation of Ca²⁺ release from mouse half-calvaria by HCO₃⁻ acidosis (produced by addition of 4, 8, 12, or 16 meq/l H⁺ to culture medium) was greater than the stimulation resulting from CO₂ acidosis (produced by raising culture medium Pco₂ to 87.2 or 108.5 mmHg) at comparable pH values. In dead bones (hatched bar), severe CO₂ acidosis was associated with a small Ca²⁺ influx; effects of HCO₃⁻ acidosis on dead bones are shown in Fig. 1. Increasing Pco₂ from 54.5 to 87.2 and 108.5 mmHg resulted in calculated HCO₃⁻ concentrations of 15.8, 21.6, and 22.5 mmol/l, respectively, whereas addition of 4, 8, 12, and 16 meq/l H⁺ to culture medium at constant Pco₂ (54.5 mmHg) yielded calculated HCO₃⁻ concentrations of 13.5, 11.3, 9.7, and 8.4 mmol/l, respectively. Ca²⁺ release values are means ± SE (n = 5). Significantly different from control: **P < 0.01; ***P < 0.001.

**Fig. 3.** Complete blockage of HCO₃⁻ acidosis-stimulated Ca²⁺ release from 3-day cultures of mouse half-calvaria by salmon calcitonin (sCT; 20 ng/ml). Pco₂ was 36.3 mmHg. Values are means ± SE (n = 5). Significantly different from control: ***P < 0.001.
served in bones treated with 10 nM 1,25(OH)₂D₃ (Fig. 8D), 20 ng/ml PTH-(1–34), or 1 μM PGE₂. However, cell counts revealed that HCO₃⁻ acidosis reduced the numbers of osteoclast-like cells by ~50% compared with controls, whereas 1,25(OH)₂D₃ treatment caused increases of ~75% (Fig. 9).

DISCUSSION

The present study correlates Ca²⁺ release data with histological evidence to demonstrate that osteoclastic resorption in cultured mouse calvarial bones is extremely sensitive to activation by HCO₃⁻ acidosis, an effect which can cause bone destruction equivalent to the maximal osteolysis produced by agents such as 1,25(OH)₂D₃, PTH, or PGE₂. Furthermore, our results show that the action of these classical osteolytic agents is attenuated markedly by slight alkalinization. We also found that severe CO₂ acidosis resulted in increased osteoclastic resorption.

Three separate lines of evidence indicate that net Ca²⁺ release into the culture medium from mouse calvarial bones stimulated by HCO₃⁻ acidosis is almost
Fig. 7. Dependence of the osteolytic action of PGE$_2$, 1,25(OH)$_2$D$_3$ and PTH on ambient acidification in 3-day cultures of mouse half-calvaria. Addition of 15 meq/l OH$^-$ as NaOH increased final medium pH from ~7.1 in control cultures to ~7.3, resulting in marked attenuation of Ca$^{2+}$ release in all treatment groups. PCO$_2$ was 57.2 mmHg. Values are means ± SE (n = 5). Significantly different from control group (C): *P < 0.05; ***P < 0.001; significantly different from respective, nonalkalized treatment group: #P < 0.05 (PTH), ###P < 0.01 (PGE$_2$).

entirely the result of osteoclast activity. First, salmon calcitonin (20 ng/ml) completely blocked the Ca$^{2+}$ release from live bones stimulated by HCO$_3^-$ acidosis. Second, in bones killed by freeze-thawing, HCO$_3^-$ acidosis exerted only a minimal effect, i.e., a slight reduction of the net Ca$^{2+}$ influx that was always observed. Although such dead bones do not provide a perfect control because freeze-thawing may render the cellular lining of calvarial surfaces more “leaky,” our data suggest that the influence of HCO$_3^-$ acidosis on physicochemical Ca$^{2+}$ exchange was small, even at large acid loads. These results are in essential agreement with the earlier findings of Goldhaber and Rabadjija (17). Third, in calvaria exposed to HCO$_3^-$ acidosis (>12 meq/l H$^+$), extensive osteoclastic resorption cavities were present; such cavities were absent in calcitonin-treated bones.

Our findings show that bone resorption in cultured mouse calvaria may be more sensitive to small pH changes than was previously appreciated. Ca$^{2+}$ release was minimal above pH 7.2 in control cultures but was stimulated strongly by the addition of small amounts of H$^+$ (i.e., HCO$_3^-$ acidosis) to culture medium. Figure 2 shows that addition of 4 meq/l H$^+$ reduced pH from 7.12 to 7.03 but increased Ca$^{2+}$ release 3.8-fold; an 11-fold increase was observed with 16 meq/l added H$^+$. The steep responses to HCO$_3^-$ acidosis evident in Figs. 1 and 2 resemble closely the acid-activation curve for resorption pit formation by cultured rat osteoclasts in which resorption is essentially “switched off” above pH 7.2 and stimulated maximally at pH ~6.9 (6, 31). Osteoclasts derived from chick long bones (4, 28), human osteoclastoma tissue (19), or long-term mouse marrow cultures (29) show remarkably similar responses to extracellular pH changes. Taken together, these observations suggest that the local pH in the microenvironment of osteoclasts in cultured mouse parietal bones is close to that of the tissue culture medium and thus considerably lower than that of blood pH (~7.40).

We also demonstrated that CO$_2$ acidosis is a less effective stimulator of Ca$^{2+}$ release from calvarial bones than HCO$_3^-$ acidosis over a similar pH range, a result that is in good agreement with data from the $^{45}$Ca$^{2+}$ flux experiments of Bushinsky and colleagues (11, 12). One partial explanation for the smaller stimulatory effect of CO$_2$ acidosis may be that hypercapnia promotes deposition of Ca$^{2+}$, as carbonates, on bone surfaces (11). Nevertheless, our own results show that resorption is activated quite strongly by severe CO$_2$ acidosis at low pH (PCO$_2$ = 108.5 mmHg; pH = 6.81). It may be that, when PCO$_2$ is increased, the “set point” at which resorption begins to be acid activated is shifted to lower pH values. It is also noteworthy that the striking stimulation of bone resorption caused by severe CO$_2$ acidosis occurred despite an increase in HCO$_3^-$ concentration (22.5 mM compared with control value of 15.8 mM). This result suggests that, in the main, it is the increased H$^+$ concentration that is ultimately responsible for acidosis-stimulated osteoclastic bone resorption. Experiments with cultured osteoclasts have not shown clear differences between the effects of CO$_2$ and HCO$_3^-$ acidosis in stimulating pit formation (Ref. 2; M. Morrison and T. R. Arnett, unpublished data). The reasons for this discrepancy are unknown. In vivo, HCO$_3^-$ acidosis is associated with bone loss (reviewed in Ref. 5). In thyroparathyroidectomized rats, acute HCO$_3^-$ acidosis induced by acid feeding results in a striking hypercalcemia that is prevented by calcitonin or colchicine, implying osteoclast involvement (22). The effects of CO$_2$ acidosis in vivo are less well investigated; however, a recent study (32) reported that hormone replacement therapy causes a respiratory alkalosis in normal postmenopausal women and that changes in blood pH were inversely correlated with those in urinary excretion of hydroxyproline, an index of bone resorption.

The behavior of the calvarial system in response to acid stimulation also differs from that of cultured osteoclasts in another key respect. We found that resorption stimulated by HCO$_3^-$ acidosis could be blocked by the cyclooxygenase inhibitors indomethacin and ibuprofen, in line with earlier findings (17, 33) and suggesting a requirement for endogenous prostaglandin synthesis. In contrast, resorption pit formation by cultured osteoclasts is stimulated by cyclooxygenase inhibitors (26, 27) and is inhibited by prostaglandins (4, 13). Our observation that HCO$_3^-$ acidosis-activated resorption was additionally attenuated by two mechanistically distinct inhibitors of the 5-lipoxygenase pathway suggests that leukotrienes, which stimulate bone resorption in a number of systems (16, 25), are also involved in mediating the effect.
In this study, we correlated a biochemical index of calvarial bone resorption (Ca\(^{2+}\) release) with quantitative histological analysis of the same bones. Surprisingly, perhaps, this approach has not been used before. The simple whole mount histological technique we used was first described by Marshall and colleagues (20, 23), who reported that ~200 multinucleate, TRAP-positive osteoclasts were visible in single parietal bones of 4-day-old mice before being placed in culture; our own data for control bones agree well with these values. In calvaria exposed to HCO\(_3\) acidosis, extensive osteoclastic resorption cavities were evident, but TRAP-positive osteoclast numbers were reduced consistently. Whether this change in osteoclast numbers reflects reductions in osteoclast survival, formation, or both, is uncertain. Cell culture experiments have shown that osteoclast formation in 10-day mouse marrow cultures is inhibited at pH 6.9–7.0 (29); however, survival of mature osteoclasts in this pH range appears unimpaired (3, 6, 31). Thus the impressive resorption

Fig. 8. Whole mount mouse half-calvaria stained to demonstrate tartrate-resistant acid phosphatase (TRAP) after 72-h culture, viewed by transmitted light microscopy. A: nonacidified control, pH 7.208, showing small resorption cavities (arrowheads); scale bar = 500 \(\mu\)m. B: bone treated with 12 meq/l H\(^+\), pH 7.01 (HCO\(_3\) acidosis); scale bar = 500 \(\mu\)m. C: higher power detail of bone exposed to severe CO\(_2\) acidosis (108.5 mmHg) at pH 6.82; osteoclasts in scalloped resorption bays (arrowheads) are clearly evident; scale bar = 100 \(\mu\)m. D: bone treated with 10 nM 1,25(OH)\(_2\)D\(_3\), pH 7.09; scale bar = 500 \(\mu\)m. Large areas of osteolysis, demonstrated by red-black TRAP staining of scalloped resorption fronts and osteoclasts (arrowheads), are visible in acidosis and 1,25(OH)\(_2\)D\(_3\)-treated bones.

Fig. 9. Stimulation of TRAP-positive osteoclast (OC)-like cell numbers in whole mount mouse half-calvaria by 10 nM 1,25(OH)\(_2\)D\(_3\) (1,25 D\(_3\)), but not 1 \(\mu\)M PGE\(_2\) or HCO\(_3\) acidosis resulting from addition of 15 meq/l H\(^+\). Significantly different from control (C): *\(P < 0.05\).
cavities seen in calvaria exposed to acidosis may reflect mainly the activation of preexisting quiescent osteoclasts rather than the formation of new osteoclasts. However, the possibility cannot be excluded that acidosis rapidly stimulates the formation of new osteoclasts, which are then immediately activated, alongside increased apoptosis of preexisting quiescent osteoclasts.

In contrast, the osteolytic effects of 1,25(OH)₂D₃ appeared to be related, at least in part, to increases in TRAP-positive osteoclast numbers, reflecting the stimulatory action of this hormone on osteoclast formation in long-term marrow cultures. Ca²⁺ release in 1,25(OH)₂D₃-treated cultures was similar to that in cultures exposed to HCO₃⁻ acidosis, but resorption on a per cell basis was ~3.5-fold lower, based on the cell counts shown in Fig. 9. Interestingly, cultures treated with 1,25(OH)₂D₃, PTH, or PGE₂ generally exhibited similar acidification compared with controls. Similar effects of PTH have been described previously (10, 14); this is probably due to a stimulation of H⁺ efflux from osteoblast-like cells derived from rodent calvaria (34). Other bone-resorbing agents reported to stimulate H⁺ efflux from osteoblast-like cells include insulin-like growth factor I (35), interleukin-1 (1, 34) and ATP (21). Obviously, such acidification could account for some of the osteolytic action of these agents (5, 30).

In conclusion, our findings indicate that the modulation of osteoclast activity by small pH changes is a key determinant of bone resorption in mouse calvarial cultures. Similar responses to extracellular acidification have been observed in all bone resorption systems examined to date. The great sensitivity of osteoclasts to extracellular protons may have evolved in vertebrates as a last line of defense against systemic acidosis.

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REFERENCES


