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Antithetic Effects of Ryanodine and Ruthenium Red on Osteoclast-Mediated Bone Resorption and Intracellular Calcium Concentrations

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Abstract In the process of bone remodeling, osteoclasts are responsible for resorption of bone. High levels of intracellular calcium decrease the bone resorbing activity of osteoclasts and increase detachment of osteoclasts from the bone surface. The regulatory role of intracellular calcium in bone resorption is not clearly understood. To understand this phenomenon, we studied the effects of the intracellular calcium modulators ryanodine and ruthenium red on bone resorption using the disaggregated osteoclast pit assay. Changes in intracellular calcium concentrations after treatment with these compounds were detected with the fluoroprobe fura2. With ryanodine, a significant, dose-dependent decrease in bone resorption was detected. This inhibition of bone resorption was reversible upon the removal of ryanodine. Ryanodine increased intracellular calcium concentrations, suggesting that the mechanism of inhibition by ryanodine was via alterations in intracellular stores of calcium. After treatment with ruthenium red, osteoclasts resorbed significantly more bone compared to vehicle-treated cells. This increase in bone resorption correlated with a decrease in intracellular calcium concentrations. The addition of parathyroid hormone or ruthenium red to osteoclast cultures containing ryanodine did not attenuate the decrease in bone resorption caused by ryanodine, suggesting that the mechanism of ryanodine inhibition of bone resorption may involve the "locking" of a calcium channel in an open position.

Key words: calcium channel, calcium modulators, disaggregated osteoclast pit assay, fura2, intracellular calcium stores, osteoclast regulation

Osteoclasts attach to mineralized surfaces and actively resorb bone, releasing calcium into the extracellular environment [Boonekamp et al., 1986]. High concentrations of extracellular calcium result in decreased bone resorbing activity and/or increased cell retraction and detachment from bone [Silver et al., 1988; Datta et al., 1989]. Recently, it has been reported that compounds that modulate calcium channel/calcium receptor function influence cytosolic calcium concentrations in the osteoclast [Zaidi et al., 1990, 1991; Datta et al., 1990; Miyauchi et al., 1990; Shankar et al., 1992a,b, 1993; Bax et al., 1992; Pazianas et al., 1992; Hammerland et al., 1994]. These conclusions are based on the observations that magnesium, barium, and lanthanum ions increase $[Ca^{2+}]_i$, suggesting that di- and trivalent cations bind to a calcium receptor and increase intracellular calcium concentrations in a manner similar to that seen with high levels of extracellular calcium. Collectively, these data suggest that a "calcium receptor" located on the osteoclast membrane regulates bone resorption by detecting increased levels of extracellular calcium concentrations and signals the release of $[Ca^{2+}]_i$ to reduce bone resorbing activity. In a previous study, the L-type calcium channel antagonists PN 200-110 and (−)202-791 decreased bone resorption and increased intracellular calcium, suggesting that these compounds blocked the release of calcium from osteoclasts [Ritchie et al., 1994]. These data substantiate the presence of a calcium receptor and/or a calcium channel on the osteoclast surface.

The exact role of extracellular and intracellular calcium in controlling osteoclast activity re-

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mains unclear. To explore the role of alterations of intracellular calcium on osteoclast function, we examined the effects of the intracellular calcium modulators ruthenium red and ryanodine on bone resorption as assessed by the disaggregated osteoclast pit assay and on intracellular calcium concentrations as determined with the fluoroprobe fura2. Ruthenium red is an inorganic dye which inhibits calcium uptake in the mitochondria and inhibits calcium release from the sarcoplasmic reticulum (SR) in skeletal and cardiac muscle [Moore, 1971; Rousseau et al., 1986]. Ryanodine is a neutral alkaloid that affects calcium release in the SR [Rousseau et al., 1987]. In skeletal muscle SR, ryanodine has been shown to have antithetical effects. When ryanodine was added to muscle cells in which the calcium channel was in an open state due to the presence of calcium, the calcium channel was locked in an open state, resulting in an increase in $[Ca^{2+}]_i$. When calcium was not present, ryanodine locked the channel in a closed state [Nelson, 1987]. Zaidi and coworkers [1992a,b] have proposed the existence of a ryanodine-sensitive endoplasm reticular membrane calcium receptor in the osteoclast.

In the presence of ryanodine (1 μM), bone resorption was significantly decreased and was associated with an increase in intracellular calcium levels. Osteoclasts exposed to ruthenium red (12 μM) resorbed significantly more bone that vehicle-treated cells. Ruthenium red treatment decreased intracellular calcium concentrations. In the presence of 12 μM ruthenium red, the ability of ryanodine to inhibit bone resorption was not altered. Previous studies have shown that parathyroid hormone (PTH) increases bone resorption in the disaggregated osteoclast pit assay, presumably by inducing osteoblasts to secrete a factor that stimulates osteoclast activity [Murrills et al., 1990]. Under our assay conditions, PTH (2 nM) significantly increased bone resorption. In the presence of PTH, ryanodine strongly inhibited bone resorption, suggesting that intracellular calcium modulators, such as ryanodine, have a more pronounced effect on osteoclast activity than hormonal stimulation.

MATERIALS AND METHODS

Reagents

Medium 199 with Earle's balanced salt solution (M199, EBSS), medium 199 with Hanks' balanced salt solution (M199, HBSS), ruthenium red, rat parathyroid hormone (rPTH), and acid phosphatase kit (No. A-387) were purchased from Sigma Chemical, St. Louis, MO. Fetal bovine serum (FBS) was purchased from HyClone, Logan, Utah. Penicillin/streptomycin and trypsin/EDTA were purchased from Gibco, Grand Island, NY. Fura2 was obtained from Molecular Probes, Eugene, OR. Ryanodine was purchased from Calbiochem Corp., La Jolla, CA.

Substrate Preparation

Transverse slices of dentine were cut from sperm whale teeth (IACUC A103-89), as previously described [Ritchie et al., 1994].

Disaggregated Osteoclast Pit Assay

The method used to disaggregate osteoclasts was the method established by Boyde and Chambers and modified by Dempster [Boyde et al., 1984; Chambers et al., 1985; Dempster et al., 1987]. Long bones (tibia and femur) were removed from 4–6-day-old rat pups which were sacrificed by cervical dislocation (IACUC A43-90) and the osteoclasts were disaggregated as previously described [Ritchie et al., 1994]. Briefly, long bones were dissected free of adhering tissue and curetted with scissors into prewarmed M199, HBSS, pH 6.8, with 10 mM HEPES containing 100 μg/ml streptomycin and 100 IU/ml penicillin. The cell suspension was triturated with a sterile plastic Pasteur pipette to disaggregate the cells. The resulting cell suspension was pipetted onto dentine slices presoaked in M199, HBSS in a 96-well culture dish at 37°C. After 60 min incubation at 37°C the slices were vigorously rinsed in M199, HBSS and transferred to 24-well dishes in M199, EBSS, 10 mM HEPES, 0.7% sodium bicarbonate, 10% FBS, streptomycin (1 μg/ml), and penicillin (100 IU/ml), pH 6.8. The slices were incubated at 37°C in 95% air, 5% CO_2 for the duration of the experiment.

Each experiment consisted of 12 vehicle-treated slices as controls and 12 slices treated with each test substance. After a 24 h incubation period, the experiments were terminated by fixing the slices in citrate:acetone:formaldehyde (26:66:8). The slices were stained for tartrate resistant acid phosphatase to determine the number of osteoclasts per slice. The osteoclasts were removed from the slices with 0.25 M NH_4OH, then stained with 1% toluidine blue.

The number of resorption lacunae or pits per slice were counted by reflected light microscopy

and expressed as the number of pits per osteoclast per slice. Pit area was determined with the BQ System IV (R&M Biometrics, Inc., Nashville, TN) using a digitizing tablet attached to an ZEOS microcomputer linked via a camera lucida to a Nikon Microphot-SA microscope (Nikon, Toyko, Japan). Pit area was expressed as the total pit area per dentine slice.

Intracellular Calcium Determinations

Intracellular calcium changes in osteoclasts were detected with ratio imaging using the fluorescent calcium indicator fura2 [Fay et al., 1985; Grynkiewicz et al., 1985]. Osteoclasts were disaggregated from the long bones of neonatal rats as described above and intracellular calcium concentrations were determined as described previously [Ritchie et al., 1994]. Briefly, cells were concentrated and resuspended in Krebs-Ringer buffer, pH 7.4 (140 mM NaCl, 5 mM KCl, 2 mM $CaCl_2$, 1.2 mM $MgSO_4$, 1.2 mM KH_2PO_4, 25 mM HEPES). Cells were pipetted onto a glass microscope slide allowed to attach 30 min, then rinsed with Krebs-Ringer. The slides were incubated with the pentaacetoxymethylester of fura2 (fura2-AM, 10 μM) for 30 min at 37°C, in the presence of 10% FBS. Cells were washed with Krebs-Ringer and maintained at 37°C in Krebs-Ringer, 10% FBS for 30 min to allow ester hydrolysis. The calcium binding form of fura2 has a maximum absorption of 335 nm while the calcium free form absorbs maximally at 365 nm. Cells were imaged with a Nikon Fluor 40 objective. Calcium measurements were made by obtaining the ratio of 340/380 fura2 fluorescence at 510 nm after excitation at 340 and 380 nm (Innovision, Research Triangle, NC).

Statistical Analyses

For osteoclast number and pit number per osteoclast, a one way analysis of variance (ANOVA) with post-hoc tests or the Student's *t*-test were used to determine the significance of various treatments vs. vehicle-treated controls. The area of pits created by untreated osteoclasts produced a skewed distribution, suggesting the necessity for nonparametric statistical analysis. Therefore, for area measurements, a Wilcoxon rank sum test was applied to determine the median total pit area/slice per experiment. An ANOVA was then used to determine differences between treatment groups and vehicle-treated controls. Unless otherwise specified, results are expressed as the mean ± SEM of triplicate experiments.

RESULTS

Isolated osteoclasts were treated with ryanodine for 24 h (Fig. 1). Ryanodine decreased bone resorption in a dose-dependent manner, significantly decreasing both the number of pits per osteoclast per slice and the total pit area resorbed. In the presence of 100 nM of ryanodine, the number of pits per osteoclast per slice was reduced by 56.7 ± 12.4%, and the total area resorbed was reduced by 47.6 ± 15.3% (number of osteoclasts/slice: control, 19.2 ± 1.3; 1 μM ryanodine, 23.1 ± 1.3; mean ± SEM). We saw no evidence of cell toxicity or detachment from the dentine slices after treatment with ryanodine or ruthenium red. There were no changes in the number of osteoclasts per slice after 24 h of ryanodine treatment, suggesting the compound had altered cell activity and not cell adhesion.

To test the potential toxicity of ryanodine, bone slices containing osteoclasts were incubated with 1.0 μM ryanodine for 24 h, then transferred to a dish of vehicle-treated control media for 24 subsequent hours. When cells were exposed to ryanodine for 12 h then transferred to vehicle-treated media for a subsequent 12 h (ryanodine/vehicle), there was no significant difference between the total area of dentine resorbed as compared to slices incubated in vehicle-treated media for two 12 h periods [(vehicle/vehicle) 67.4 ± 11.6 $\mu m^2 \times 10^3$ for ryanodine/vehicle vs. 104.0 ± 23.1 $\mu m^2 \times 10^3$ for vehicle/vehicle]. Significant differences in inhibition of resorption were attained among both the vehicle/vehicle and the ryanodine/vehicle groups and the group treated for both 12 h periods with ryanodine (ryanodine/ryanodine group, total area resorbed 7.9 ± 1.6 $\mu m^2 \times 10^3$, $P < 0.05$ as compared to vehicle/vehicle or ryanodine/vehicle, Fig. 2). Osteoclasts recovered from ryanodine inhibition after 24 h of ryanodine-free media, suggesting that the mechanism of action of ryanodine was not harmful to osteoclast function.

In addition, it should be noted that ryanodine treatment significantly decreased the average size of the resorption lacanue. For the vehicle-treated control, the average pit area was 12.4 ± 1.0 $\mu m^2 \times 10^3$ compared to 3.78 ± 0.2 $\mu m^2 \times 10^3$ after treatment with 1 μM ryanodine ($P = 0.004$).

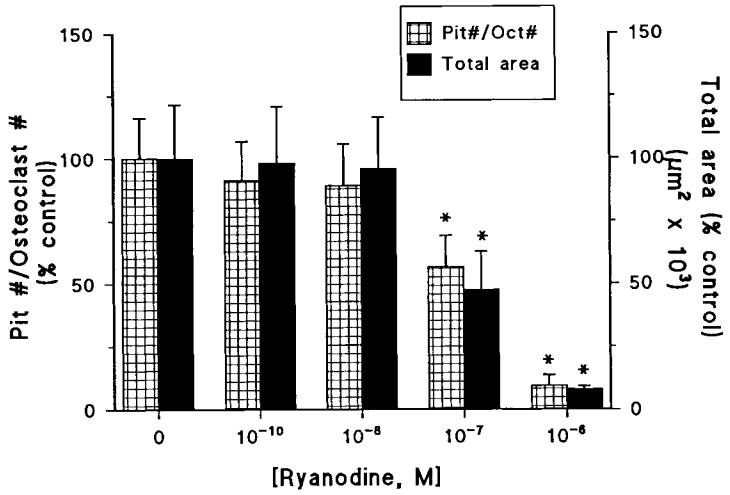

Fig. 1. Dose response of ryanodine on bone resorption. Dentine slices were incubated for 24 h with osteoclast enriched cultures in the presence of 0, 0.1 nM, 10 nM, 100 nM, or 1 μM ryanodine. Results are expressed as the number of pits/osteoclast/slice and as total pit area resorbed and were determined by the disaggregated pit assay. Data are the mean ± SEM of duplicate experiments. *$P < 0.05$ by ANOVA with Bonferroni's correction.

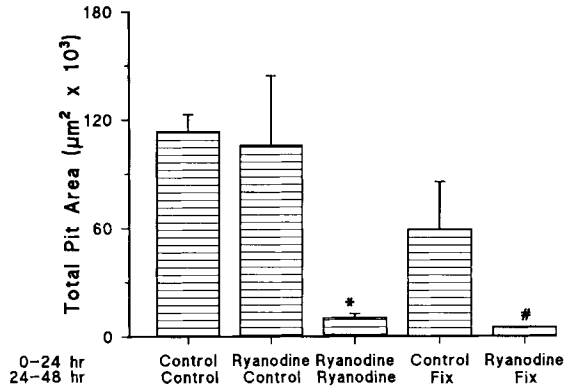

Fig. 2. Reversibility of inhibition by ryanodine on bone resorption. Osteoclasts attached to dentine slices were divided into 5 groups and incubated for 24 h. Three groups were incubated with ryanodine at 1 μM and two groups with vehicle-treated control. One set of each group were fixed after 24 h (vehicle/fix, ryanodine/fix). The remaining slices were transferred to either vehicle-treated control media or media containing 1 μM ryanodine (vehicle/vehicle, ryanodine/vehicle, ryanodine/ryanodine). Data are expressed as the total area of dentine resorbed. Results are of average of two experiments, mean ± SEM. *$P < 0.05$ as compared to vehicle/vehicle, #$P < 0.05$ as compared to vehicle/fix by Student's t-test.

The addition of ryanodine increased intracellular calcium concentrations in individual osteoclasts. Addition of 5 μM ryanodine to osteoclasts loaded with fura2 increased the intracellular calcium concentration 2-fold over the baseline (Fig. 3).

Ruthenium red increased bone resorption in a dose-dependent manner, significantly altering the number of pits per slice at 12 μM (200.0 ± 33.6% of vehicle-treated control) and the total area resorbed at 24 μM (269.2 ± 35.4% of vehicle-treated control, Fig. 4). When osteoclasts loaded with fura2 were exposed to 5 mM $CaCl_2$, intracellular calcium increased 3-fold over the baseline. Addition of 60 μM ruthenium red to these cells decreased intracellular calcium concentrations to baseline levels (Fig. 5).

Ruthenium red treatment had no effect on the number of osteoclasts/slice (control, 10.5 ± 1.3; 24 μM ruthenium red, 11.9 ± 1.0 osteoclasts/slice), nor did ruthenium red have any effect on the average pit area. Average pit area of control slices was 10.3 ± 1.9 $\mu m^2 \times 10^3$ and after treatment with 24 μM ruthenium red, average pit area was 10.6 ± 1.4 $\mu m^2 \times 10^3$.

To ascertain if ryanodine was a competitive inhibitor of ruthenium red-stimulated bone resorption, disaggregated osteoclasts were assayed in the presence of vehicle, ruthenium red, ryanodine, or both ruthenium red and ryanodine. Ruthenium red (12 μM) significantly increased the total pit area (148.3 ± 24.7% of vehicle-treated control). Ryanodine (1 μM) significantly decreased the total pit area resorbed (6.5 ± 2.3% of vehicle-treated control, $P < 0.05$). When assayed together, ruthenium red had no effect on ryanodine inhibition of bone resorption, suggesting that the compounds do not compete at the same extra- or intracellular level or change intracellular signals (Fig. 6). Ruthenium red was unable to prevent the decrease in aver-

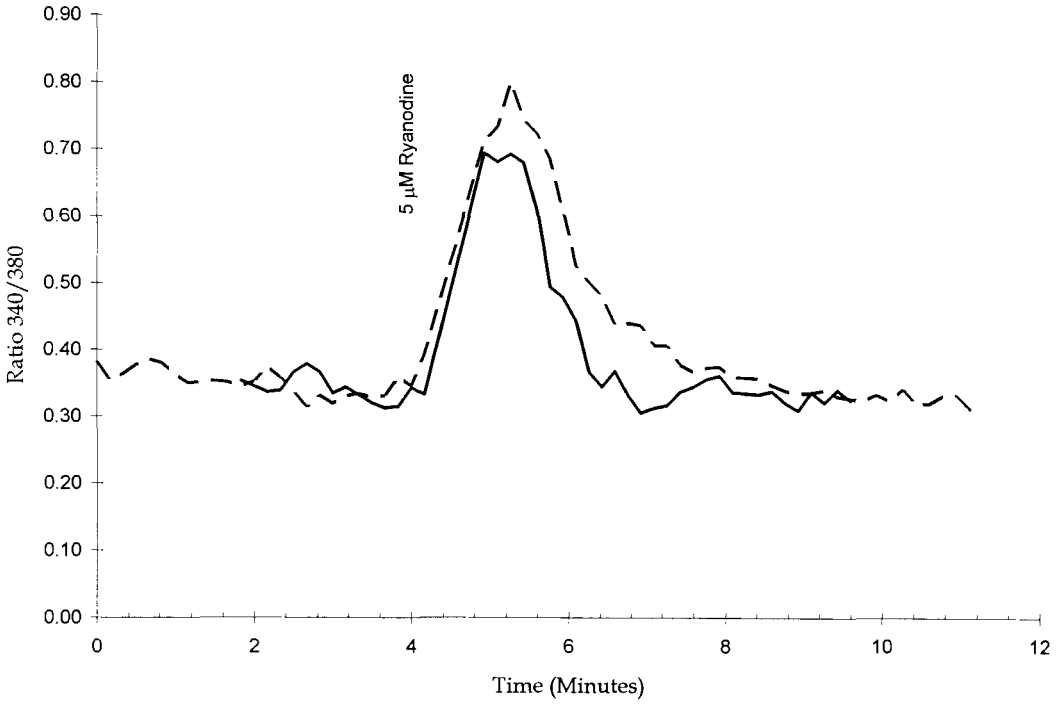

Fig. 3. The effect of ryanodine on intracellular calcium levels. Intracellular calcium concentrations were determined by imaging with fura2. Osteoclasts were attached to glass microscope slides and loaded with 10 μM fura2. Intracellular calcium concentrations were determined by measuring the ratio of fluorescence at 380/340. Addition of 5 μM ryanodine increased intracellular calcium 2-fold over the baseline. The dotted line and the solid line each represent separate cells from separate experiments.

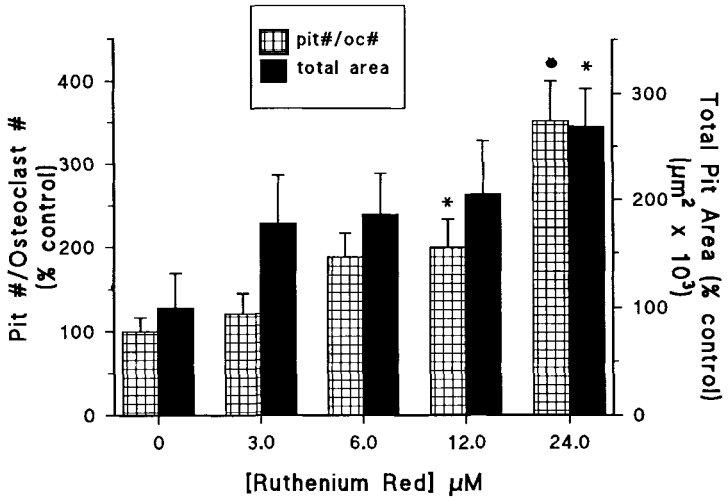

Fig. 4. Dose response of ruthenium red on bone resorption. Dentine slices were incubated for 24 h with osteoclast enriched cultures in the presence of 1, 3, 6, 12, 24, or 60 μM ruthenium red. Results are expressed as the number of pits/osteoclast/slice, and the total area resorbed was determined by the disaggregated osteoclast pit assay. Results are the mean ± SEM of duplicate experiments. *$P < 0.05$ by ANOVA with Bonferroni's correction.

age pit area caused by ryanodine treatment (data not shown).

PTH (2 nM) also had no effect on reversing inhibition of bone resorption by ryanodine (Fig. 7). Disaggregated osteoclasts were incubated with vehicle, ryanodine (1 μM), PTH (2 nM), or PTH and ryanodine. Ryanodine significantly decreased the total pit area resorbed (15.1 ± 6.1% of vehicle-treated control) and PTH significantly increased the total pit area (163.9 ± 24.4%

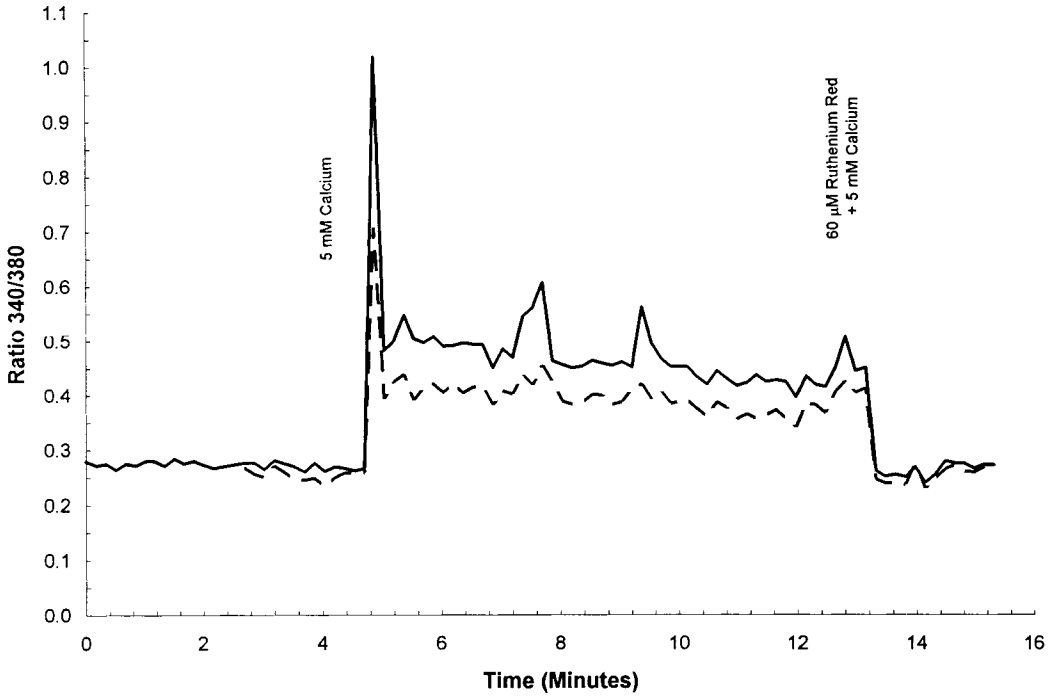

Fig. 5. The effect of ruthenium red on intracellular calcium. Intracellular calcium concentrations were determined by fura2 fluorescence. Osteoclasts were attached to glass microscope slides and loaded with 10 μM fura2. Intracellular calcium concentrations were determined by measuring the ratio of fluorescence at 380/340. Addition of 5 mM calcium increased intracellular calcium 3-fold over the baseline, addition of 60 μM ruthenium red returned the intracellular concentration of calcium to baseline levels. Lines (*dotted and solid*) represent two separate cells from separate experiments.

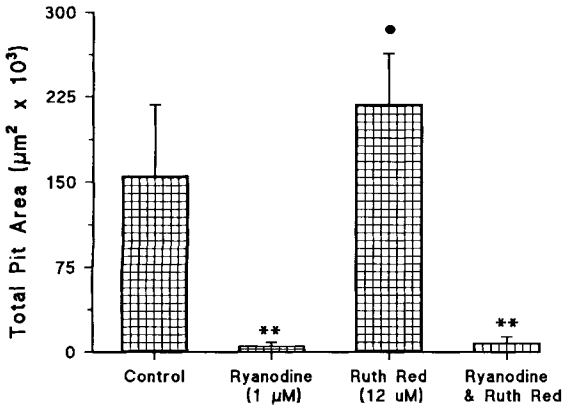

Fig. 6. The effect of ruthenium red on inhibition of bone resorption by ryanodine. Dentine slices were incubated for 24 h with osteoclast-enriched cultures in the presence of vehicle, 1 μM ryanodine, 12 μM ruthenium red or ryanodine (1 μM), and ruthenium red (12 μM). Results are expressed the median ± 95% confidence level of the total area resorbed from a representative experiment, as determined by the disaggregated osteoclast pit assay. $*P < 0.05$, $**P < 0.001$ as compared to vehicle-treated control by Wilcoxon rank sum.

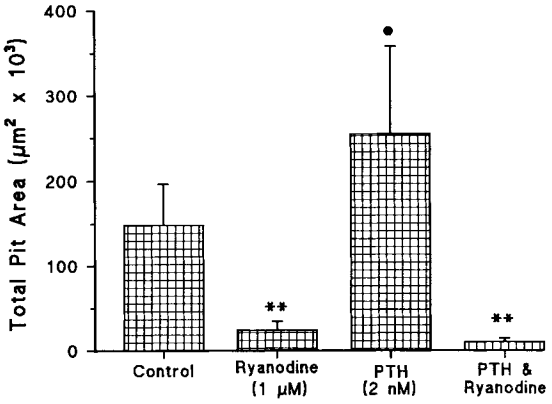

Fig. 7. The effect of PTH on inhibition of bone resorption by ryanodine. Dentine slices were incubated for 24 h with osteoclast enriched cultures in the presence of vehicle, 1 μM ryanodine, 2 μM PTH or ryanodine (1 μM), and PTH (2 nM). Results are expressed as the median ± 95% confidence level of the total area resorbed of a representative experiment, as determined by the disaggregated osteoclast pit assay. $*P < 0.05$, $**P < 0.001$ as compared to control by Wilcoxon rank sum.

over control). There was no change in inhibition by ryanodine with the addition of PTH to the culture (ryanodine alone: 15.1 ± 6.1% of vehicle-treated control; ryanodine and PTH: 11.8 ± 3.2% of vehicle-treated control). PTH was unable to prevent the decrease in average pit area caused by ryanodine treatment (data not shown).

DISCUSSION

The importance of calcium homeostasis in skeletal growth and remodeling cannot be underestimated. Osteoclasts utilize a classic mechanism of feedback inhibition to control bone resorption. Osteoclasts attach to bone and resorb the mineralized matrix, releasing calcium into the extracellular environment. High extracellular calcium concentrations increase the level of $[Ca^{2+}]_i$ thereby decreasing bone resorption. Modulators of $[Ca^{2+}]_i$, such as ryanodine and ruthenium red, affect this tight interactive control of calcium in the osteoclast and alter the resorptive capabilities of the osteoclast.

Ruthenium red increased the activity of osteoclasts by increasing the number and area of pits in the disaggregated osteoclast pit assay. This increase in bone resorption correlated with a decrease in intracellular calcium in osteoclasts. In skeletal and cardiac muscle, ruthenium red decreased calcium uptake in the mitochondria and inhibited calcium release from the sarcoplasmic reticulum [Moore, 1971; Rousseau et al., 1987]. Ruthenium red is an inorganic tetramer that is unable to penetrate cells. Although in this present study we did not elucidate the definitive mechanism by which ruthenium red stimulates bone resorption, the data suggest that ruthenium red may alter a calcium receptor on the surface of the osteoclast membrane, prohibiting calcium uptake and allowing for the efflux of calcium from the cell.

The plant alkaloid ryanodine significantly decreased bone resorption in a dose-dependent manner in the disaggregated osteoclast pit assay. The ability of osteoclasts to recover from the inhibition of ryanodine and return to a resorptive state suggests that the binding of ryanodine to a calcium channel, calcium receptor, or a ryanodine receptor is reversible. In this in vitro system, the inhibition of bone resorption by ryanodine could be due to cell death. In order to test the toxicity of these compounds, osteoclasts were incubated with ryanodine for 24 h and then returned to media without ryanodine for an additional 24 h. No significant differences were noted among these groups and control groups treated with the vehicle for both 24 h periods. These data demonstrate that the inhibition of pit formation by these modulators of intracellular is not due to toxic effects on the osteoclast and is reversible. We saw no evidence of cell toxicity or detachment from the dentine slices after treatment with the either ruthenium red or ryanodine, suggesting that our compounds altered cell activity rather than cell attachment.

Ryanodine increased $[Ca^{2+}]_i$ 2-fold over baseline levels in rat osteoclasts. Zaidi and coworkers showed ryanodine to be a voltage-dependent uncoupler of the osteoclast calcium receptor [Zaidi et al., 1992a]. Rat osteoclasts were loaded with fura2 in a serum-free, calcium-free, ethyleneglycol bis-(aminoethyl ether) tetraacetic acid (EGTA) containing buffer, and $[Ca^{2+}]_i$ concentrations were determined. The authors saw a monophasic elevation in $[Ca^{2+}]_i$ with the addition of Ni^{2+} and valinomycin. This increase in $[Ca^{2+}]_i$ was eliminated by the addition of 10 μM ryanodine.

In our study, ryanodine treatment increased $[Ca^{2+}]_i$ in fura2 loaded rat osteoclasts. One possible explanation for these seemingly disordant results among studies are differences in the imaging conditions. In our study, osteoclasts were loaded and assayed in the presence of 10% FBS in Krebs-Ringer HEPES buffer. This buffer contained 2 mM Ca^{2+} and 6.2 mM K^+; no EGTA, which will chelate cations, was present. Other investigators have noted discrepancies in the actions of ryanodine on skeletal and cardiac muscle. Nelson noted that ryanodine locked open a calcium channel in the presence of calcium and locked the channel closed in the absence of calcium [Nelson, 1987]. If this theory is applied to osteoclasts, it explains the discrepancies between previously published work [Zaidi et al., 1992a] and this study. The previous work was performed under calcium-free conditions and it is feasible that ryanodine locked the calcium channel/receptor into a closed state, whereas in this present study there was adequate calcium present available upon addition of ryanodine to lock the calcium channel/receptor in an open state. This hypothesis explains both the increase in $[Ca^{2+}]_i$ and the decrease in bone resorption noted after ryanodine treatment in this study.

Further evidence for the ability of ryanodine to lock open a calcium channel can be found in the lack of ability of PTH and ruthenium red to alter inhibition of bone resorption by ryanodine. Both PTH and ruthenium red were potent stimulators of bone resorption in the disaggregated osteoclast pit assay in this study. The inability of either compound, one of which affects $[Ca^{2+}]_i$ (ruthenium red), the other which acts by stimulation by a yet unidentified osteoblastic factor (PTH) to overcome ryanodine inhibition suggests the possibility that ryanodine is functioning, under our assay conditions, to lock the calcium channel in an open state.

Osteoclasts, while resorbing bone, are motile and dissolve the matrix as they travel along the bone surface. Increases in $[Ca^{2+}]_i$ decreased bone resorption, reducing both the attachment and enzymatic activity of osteoclasts. Ryanodine decreased the size of resorption lacunae, even in the presence of ruthenium red or PTH. In this study, both PTH and ruthenium red stimulated resorption by increasing the production of pits, rather than the size of lacunae. In contrast, ryanodine reduced bone resorption by decreasing not only the production of pits, but also the average size of the pits. This suggests that ryanodine causes an influx of calcium into the osteoclasts and lends further evidence for ryanodine locking open a calcium channel.

The underlying mechanism for regulation of osteoclasts by calcium remains to be elucidated. The majority of the literature to date has suggested the presence of either a calcium channel or a calcium receptor on the osteoclast. Although our study does not rule out the possibility of a calcium receptor, our data support the presence of a calcium channel. There is convincing evidence for both a calcium receptor and/or a calcium channel on the osteoclast. Recent studies have provided evidence for the presence of an osteoclast "calcium receptor" on the osteoclast membrane [Zaidi et al., 1991; Shankar et al., 1992a,b, 1993; Bax et al., 1992; Pazianas et al., 1992]. This concept was demonstrated by the ability of polyvalent ions such as barium, magnesium, nickel, and lanthanum to increase $[Ca^{2+}]_i$, presumably by binding to a calcium receptor and inducing an increase in intracellular calcium similar to that seen with high levels of extracellular calcium.

Avian osteoclasts respond to treatment with BAY K 8644, a calcium channel agonist, by increasing $[Ca^{2+}]_i$ [Miyauchi et al., 1990], suggesting that BAY K 8644 stimulated Ca^{2+} influx through dihydropyridine-sensitive voltage-gated calcium channels. We recently showed that the L-type dihydropyridine sensitive calcium channel antagonists, PN 200-110 and (−) 202-791 inhibit bone resorption and increase intracellular levels in rat osteoclasts [Ritchie et al., 1994]. The data presented here suggests that in the presence of ryanodine, calcium channels are locked in an open position and allow influx of calcium. These studies lend further credence for the existence of a calcium channel either existing separately or in combination with a calcium receptor.

ACKNOWLEDGMENTS

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