COMMENTARY

Mechanisms of calcium disposal from osteoclastic resorption hemivacuole

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Abstract

One of the most remarkable but neglected aspects of osteoclast function is its unique adaptation that allows the cell to function despite its resorbing surface being exposed to extremely high levels of ambient Ca\(^{2+}\). Recently our studies have provided evidence of continuous transcellular Ca\(^{2+}\) disposal, suggesting that osteoclasts are able to prevent Ca\(^{2+}\) accumulation within the resorptive hemivacuole. It has also been shown that matrix protein degradation products that accumulate within the osteoclast resorptive vacuole are also undergoing transcellular transport by transcytosis. However, both experimental evidence and theoretical considerations suggest that transcellular transport of Ca\(^{2+}\) and matrix protein is likely to occur via distinct routes. In light of these considerations, we are able to provide convincing explanations for the apparent anomalies of osteoclast intracellular [Ca\(^{2+}\)] responses to a variety of endocrine stimuli. The understanding of the mechanisms involved in Ca\(^{2+}\) handling by osteoclasts indicates the lack of a simple link between osteoclast function and changes in overall cytosolic [Ca\(^{2+}\)].


Introduction

Osteoclasts are multinucleate cells that play a critical role in bone morphogenesis and remodelling. A number of metabolic bone diseases arise due purely to a net increase in osteoclastic activity; the increase in activity may be subtle but insidious, as in osteoporosis, or acute and aggressive, as in hypercalcaemia of malignancy. Despite its central importance in the pathogenesis of a number of metabolic bone diseases, many aspects of osteoclast function remain unclear. During bone resorption, a large amount of Ca\(^{2+}\) is generated within the osteoclast resorptive hemivacuole and [Ca\(^{2+}\)] in the resorptive hemivacuole can reach up to 40 mM (Silver et al. 1988). The precise mechanisms involved in the disposal of Ca\(^{2+}\) are not clear. Understanding the mechanisms of Ca\(^{2+}\) disposal is of immense importance as it may lead to the development of novel therapeutic strategies for inhibiting excessive osteoclast resorptive activity.

In order to address this problem we used an in vitro model system that employed scanning electrochemical microscopy and transparent resorbable matrices that closely mimic bone-resorbing osteoclast in vivo. This model system demonstrated that the Ca\(^{2+}\) produced in the resorption hemivacuole by the osteoclast resorptive activity was continually transported out of the resorptive site (Berger et al. 1999, 2001). These in situ studies have also suggested that in a bone-resorbing osteoclast a relatively large amount of calcium enters from the resorption hemivacuole into the cell and is released in a constant steady-state manner at the basolateral surface (Berger et al. 2001). However, the nature of the mechanisms and structures that are involved in the transport of Ca\(^{2+}\) from the hemivacuole to the basolateral surface are not known.

Routes of Ca\(^{2+}\) disposal

In the light of recent observations of continuous disposal of Ca\(^{2+}\) from the resorptive site (Berger et al. 2001) and the demonstration of bulk trafficking of matrix protein collagen by transcytosis (Nesbitt & Horton 1997, Salo et al. 1997), we have considered the possible routes of Ca\(^{2+}\) disposal from osteoclast hemivacuole. The likely routes are as follows.

(1) Bulk transport by transcytosis, i.e. where the osteoclast acts as a conduit allowing the calcium to pass as a part
of the bulk flow in manner akin to the one described as ‘trafficking’ of collagen matrix (Nesbitt & Horton 1997, Salo et al. 1997).

(2) Selective uptake involving specialised Ca$^{2+}$ channels in the cell plasma membrane at the site of the resorptive vacuole and involving intracellular channels traversing the cell and opening at the ventral surface. The release of the calcium could be facilitated by the presence of Ca$^{2+}$ pumps at the basolateral surface, thereby accelerating calcium disposal at the surface.

(3) Ca$^{2+}$ may in fact ‘leak’ around the sealing zone of the resorptive hemivacuole, though this seems inconsistent with the requirement for the osteoclast to form a tight seal to create a local microenvironment conducive to initiating resorption.

It is also possible that all the above-mentioned mechanisms (1–3) may operate, making variable contributions to the Ca$^{2+}$ disposal process. Here we consider experimental evidence and theoretical arguments to demonstrate that the ‘selective’ rather than the ‘bulk transcytosis’ or ‘leakage’ is likely to be the main mode of Ca$^{2+}$ disposal. We believe that possibility (3) is the least likely since the generation of a low pH in the hemivacuole to initiate dissolution of the inorganic component of the matrix would be frustrated by the rapid diffusion of protons out of the hemivacuole. It is worth noting that the diffusion coefficient of protons in aqueous media ($9.3 \times 10^{-5}$ cm$^2$/s) is much larger than that of any other small ion, e.g. Ca$^{2+}$ ($7.9 \times 10^{-6}$ cm$^2$/s).

Experimental evidence for selective transport

Interestingly, fluorescence microscopic experiments have indicated transport of collagen through the osteoclast, a process termed transcytosis, during resorption (Nesbitt & Horton 1997, Salo et al. 1997). These studies also provided evidence for the transcytosis of the inorganic phosphate, thereby suggesting one possible mechanism for the disposal of Ca$^{2+}$ within the osteoclast hemivacuole. However, a close analysis of the data on the kinetics of Ca$^{2+}$ disposal at the surface of the bone-resorbing osteoclast and its comparison with fluorescence microscopic experiments reveals some critical differences between the mechanism of Ca$^{2+}$ disposal (Berger et al. 1999, 2001) and transcytosis (Nesbitt & Horton 1997, Salo et al. 1997). Perhaps the most important and obvious difference is that, unlike collagen trafficking that occurs after hours, Ca$^{2+}$ flux at the basolateral surface is seen within minutes following the ‘seeding’ of osteoclast on bone and occurs at an approximately constant rate.

In cases (1) and (3) stated above, the flow would be expected to take place continuously during the course of the resorptive process or may occur in an intermittent fashion where a quantum of calcium is transferred at some fixed intervals. In the case where specific Ca$^{2+}$ channels and pumps are involved, the most likely mode of functioning is continuous rather than intermittent disposal. Indeed, our recent studies have suggested a continuous Ca$^{2+}$ disposal via a transcellular route occurring at the surface of bone-resorbing osteoclast. The data also show Ca$^{2+}$ disposal from basolateral surface that can be detected within minutes of resorption, favouring an explanation based on the ‘selective’ route rather than ‘bulk trafficking’ or ‘leakage’. However, our data do not rule out a quantal disposal where the size of the quanta may be small and we see a net flux averaged over many quanta and also broadened in time by the diffusional blurring as the calcium transits between the basolateral surface and the microelectrode detector.

Finally, ‘bulk’ flow of Ca$^{2+}$ disposal by transcytosis would involve cytosolic vacuoles traversing the osteoclast laden with high concentration of the cation. Such exceptionally high vacuolar [Ca$^{2+}$] have not been reported, and are likely to be extremely hazardous to the cell.

Lessons from other cells

There are various documented anomalies and peculiarities of Ca$^{2+}$ handling by osteoclasts, such as erratic and inconsistent hormone-induced intracellular [Ca$^{2+}$] mobilisation, heterogeneity in hormone-induced Ca$^{2+}$ signals, high resting [Ca$^{2+}$], and rather active Ca$^{2+}$ surface efflux (Zaidi et al. 1990, MacIntyre et al. 1991, Bizzari et al. 1994, Rathod et al. 1995, Berger et al. 2001). We here propose a hypothesis that is based on recent findings in other cell types and readily explains these anomalies and peculiarities (Berridge 1993, Parekh & Penner 1997, Zhang et al. 1999, Mogami et al. 2000, Park et al. 2000). One such interesting observation is the role of endoplasmic reticulum (ER) as a functional pool for Ca$^{2+}$ in pancreatic acinar cells, which shows that ER is one functionally continuous unit, providing a homogeneous environment for the lumenal Ca$^{2+}$ concentrations (Mogami et al. 2000). The transcellular Ca$^{2+}$ transport in acinar cells is thought to involve uptake through store-operated Ca$^{2+}$ channels (SOC) (Parekh & Penner 1997) in the basal membrane and is pumped into the basal ER by Ca$^{2+}$ ATPases (Mogami et al. 2000). Ca$^{2+}$ then diffuses from the base to the apical region of the ER lumen, where it is believed to be released into cytosol via specific Ca$^{2+}$ channels (Mogami et al. 2000, Park et al. 2000). Plasma membrane Ca$^{2+}$ ATPases, concentrated in the apical membrane, then pump Ca$^{2+}$ into the apical lumen. Interestingly, such regional-specific specialisation of Ca$^{2+}$ stores has been observed in parotid acinar cells, where ryanodine- and cyclic ADP-ribose (cADPR)-sensitive stores are localised in the basal region whilst inositol triphosphate (IP$_3$)-sensitive stores are present mainly in the apical pole (Møller et al. 1996).

Of particular relevance to the Ca$^{2+}$ handling by osteoclasts is the observation that Ca$^{2+}$ released from the apical...
ER terminal is quickly replenished from the ER at the base of the acinar cells. This observation leads us to propose the existence of a qualitatively similar, but much more active and possibly larger ER network in osteoclasts that allows rapid transcellular transport of Ca$^{2+}$ from resorptive hemivacuoles to the basolateral surface (Fig. 1). The fact that acinar cells and osteoclasts have a number of common structural and functional features provides further support for our hypothesis. Just like the pancreatic acinar cell, the osteoclast is structurally and functionally polarised (Salo et al. 1997). Both cells have an extensive rough ER dominating the basolateral region, and the whole of the ER in the acinar cell is functionally connected (Glowacki et al. 1986, Park et al. 2000). The resorptive surface in resorbing osteoclasts, just as the apical region in the acinar cells, has no ER as the space is densely packed with secretory granules. However, subtle differences between osteoclasts and the acinar cells exist, such as in the nature and distribution of Ca$^{2+}$ channels involved in the uptake of Ca$^{2+}$ from the hemivacuole.

In non-excitable and excitable cells, an increase in [Ca$^{2+}$], results from the inflow of the cation through the plasma membrane. The influx of Ca$^{2+}$ into the cells takes place through three types of Ca$^{2+}$ channels, voltage-operated Ca$^{2+}$ channels (VOCCs), ligand-gated non-specific Ca$^{2+}$ channels and receptor-linked Ca$^{2+}$ channels (RLCCs) (Barritt 1999). In non-excitable cells, an additional source for the increase in [Ca$^{2+}$], is the release of Ca$^{2+}$ from the ER, whereas in excitable cells it comes from the sarcoplasmic reticulum though ryanodine receptor (RyR) Ca$^{2+}$ channels.

The osteoclast plasma membrane expresses VOCC (Teti et al. 1989, Bizzari et al. 1994), RLCC (Bennett et al. 2001), Na$^+$/Ca$^{2+}$ exchanger (Moonga et al. 2001) and a stretch-activated Ca$^{2+}$ entry pathway (Wiltink et al. 1995). Interestingly, a number of past observations have suggested osteoclast plasma membrane expressions of RyR, normally found in the sarcoplasmic reticulum in excitable cells, such as skeletal muscle. Indeed, recent studies have demonstrated that osteoclast plasma membranes express type 2
influx of Ca\textsuperscript{2+} at the resorptive site, balanced by the
between acute changes in osteoclast functional modalities
(2001). This implies the lack of a simple general association
Parkinson et al. 1991, Bizzari et al. 1994, Rathod et al. 1995, Kajiya et al. 2000, Berger et al. 2001). Indeed, an increase in [Ca\textsuperscript{3+}], has been generally accepted as a potential mechanism by which various agents inhibit osteoclastic activity. However, an elevation in [Ca\textsuperscript{2+}], is not always associated with the inhibition of osteoclasts, instead it may lead to activation, as seen following exposure to parathyroid hormone (PTH), ionomycin and pertussis (Rathod et al. 1995, Berger et al. 2001). Furthermore, physiological stimulators of osteoclasts may produce variable changes in [Ca\textsuperscript{3+}], e.g. PTH has been shown to increase, decrease or have no effect on cytosolic calcium (Rathod et al. 1995, Miyauchi et al. 1990, Berger et al. 2001). A closer scrutiny of the published literature has revealed an unusual association between [Ca\textsuperscript{2+}], and osteoclastic activity, namely that an elevation of [Ca\textsuperscript{2+}], in osteoclasts can be stimulatory, inhibitory or without effect (Miyauchi et al. 1990, Zaidi et al. 1990, MacIntyre et al. 1991, Bizzari et al. 1994, Rathod et al. 1994, 1995, Parkinson et al. 1998, Kajiya et al. 2000, Berger et al. 2001). This implies the lack of a simple general association between acute changes in osteoclast functional modalities and elevation in cytosolic calcium.

In an active osteoclast there is likely to be a continuous influx of Ca\textsuperscript{2+} at the resorptive site, balanced by the process of efflux of the cation at the basolateral surface. The dynamic balance between influx and efflux is likely to be disturbed both by the inhibitors and stimulators, such as CT and PTH respectively (Rathod et al. 1995, Berger et al. 2001). Therefore, an imbalance between influx and efflux brought about by both inhibitors and stimulators can result in a net increase in cytosolic [Ca\textsuperscript{2+}] unless both efflux and influx inhibition occurs equally and simultaneously. It should be remembered that the osteoclasts cultured on non-resorbable matrices continue to be active, as exemplified by the presence of an active proton pump and free radical generation, albeit at lower levels. These considerations suggest that dynamic efflux and influx from the osteoclast will occur but at a lower rate than seen on resorbable matrices. The spatial and temporal aspects of the inhibition and stimulation of efflux and influx of Ca\textsuperscript{2+} are equally applicable to osteoclasts cultured on resorbable and non-resorbable matrices. Since those inhibitors that act first on the basolateral surface, such as CT and IL-4, are likely to inhibit efflux before influx is abolished, they will lead to transient elevations in transit calcium in the osteoclast (Fig. 1). Indeed, experimental data demonstrate that the exposure of osteoclasts cultured on glass matrix to CT and IL-4 is found to produce consistent elevation in cytosolic Ca\textsuperscript{2+} (Bizzari et al. 1994, Berger et al. 2001). However, inhibitors that can have an instantaneous effect on the influx and efflux due to their rapid diffusion, e.g. nitric oxide, will be expected to have no effect on the in-transit Ca\textsuperscript{2+} (Park et al. 2000) (Fig. 1). Similar considerations apply to the stimulators, e.g. PTH, where our experimental data show a rise, fall or no effect on the osteoclast cytosolic Ca\textsuperscript{2+}, the variety of responses produced being determined by the extent and kinetics of stimulation of efflux and influx by the particular agent (Zaidi et al. 1990, MacIntyre et al. 1991, Bizzari et al. 1994, Rathod et al. 1994, 1995, Parkinson et al. 1998, Kajiya et al. 2000, Berger et al. 2001). The above arguments are valid and applicable both for bulk transcytosis as well as to selective uptake involving specialised Ca\textsuperscript{2+} channels. The proposed model is also consistent with experimental data demonstrating increases in osteoclast cytosolic [Ca\textsuperscript{2+}] by diverse non-endocrine agents and stimuli (Fig. 1).

In conclusion, recent evidence suggests that relatively large amounts of ionised calcium within the osteoclast hemivacuole, released from the bone matrix by osteoclastic resorptive activity, is disposed of continuously in a steady-state manner. We argue that there are likely to be three routes of cation disposal, namely ‘leak’, bulk transcytosis and ‘selective’ disposal involving Ca\textsuperscript{2+} channels and pumps. The experimental evidence and theoretical considerations suggest that the ‘selective’ route is likely to be the most important or even the only route of disposal. In views of recent observations regarding acinar cell ER in Ca\textsuperscript{2+} handling and the fact that osteoclasts and acinar cells share a number of common structural and functional features, we propose a hypothetical model for the ‘selective’ route of Ca\textsuperscript{2+} disposal from osteoclast hemivacuole. The proposed hypothetical model is able to reconcile the apparent anomalies of osteoclast [Ca\textsuperscript{2+}], responses to a variety of endocrine stimuli.

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