EXPLORING MECHANICAL LOADING INDUCED CA^{2+} OSCILLATIONS IN OSTEOCYTES

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INTRODUCTION

Bone is highly sensitive to alterations in its mechanical environment. In fact, extremely brief loading sessions (e.g., 100 s of exogenous loading) are sufficient to activate mechanosensory osteocytic cells within minutes and presumably drive osteoblastic bone formation days and weeks later[1]. One potential signaling pathway that would satisfy these observations is the Ca^{2+} ion second messenger system, which permits real-time cell responses to a variety of agonists, including mechanical stimuli[2,3]. However, given the inaccessibility of osteocytes, it is not possible to confirm this mechanism *in vivo*. As a potential solution, we have developed a novel agent based model of real time Ca^{2+} signaling. We examined the validity of the model by simulating Ca^{2+} oscillations induced in osteocytic cells challenged by either cyclic or rest-inserted mechanical stimuli.

METHODS

Cytosolic Ca^{2+} oscillations induced in osteocytic cells by mechanical stimuli were simulated in an agent based modeling environment (Netlogo 2.1). Ca^{2+} oscillations were governed by the following cellular functions previously identified *in vitro*[2,4]: 1) at steady state, Ca^{2+} influx occurs at a low-rate from the extracellular milieu, 2) for homeostasis, the resulting cytosolic Ca^{2+} build-up is removed via a concentration dependent efflux across the plasma membrane and uptake into the cell mitochondria, 3) mechanical stimuli causes a strain dependent influx of Ca^{2+} (via stretch activated Ca^{2+} channels), 4) when cytosolic Ca^{2+} concentrations exceed thresholds, endoplasmic reticulum (ER) Ca^{2+} stores are released into the cytosol, 5) the threshold for Ca^{2+} release from ER is inversely related to ER Ca^{2+} store concentrations, 6) the rate of Ca^{2+} release from the ER is strain and concentration dependent, and lastly, 7) when cells are quiescent, ER Ca^{2+} stores are replenished from the mitochondria in a concentration dependent manner. Using this model, Ca^{2+} oscillations induced by 500 s of cyclical (1-Hz) and rest-inserted stimuli (10-s rest between load cycles) were contrasted.

RESULTS AND DISCUSSION

Real-time Ca^{2+} oscillations induced by cyclic loading followed one of three fundamental responses (Fig 1a). A small subset of cells were non-responsive (~20%), while a majority of cells displayed either a high-magnitude transient Ca^{2+} oscillation followed by low-level steady state fluctuations (40%), or an additional secondary Ca^{2+} transient upon cessation of the stimulus (40%). The number of responsive cells was increased when the magnitude of the stimulus was increased, regardless of the waveform (Fig 1b). In contrast to the response to cyclic stimuli, multiple high-magnitude Ca^{2+} oscillations were induced when cells were subjected to rest-inserted stimuli (Fig 1c).

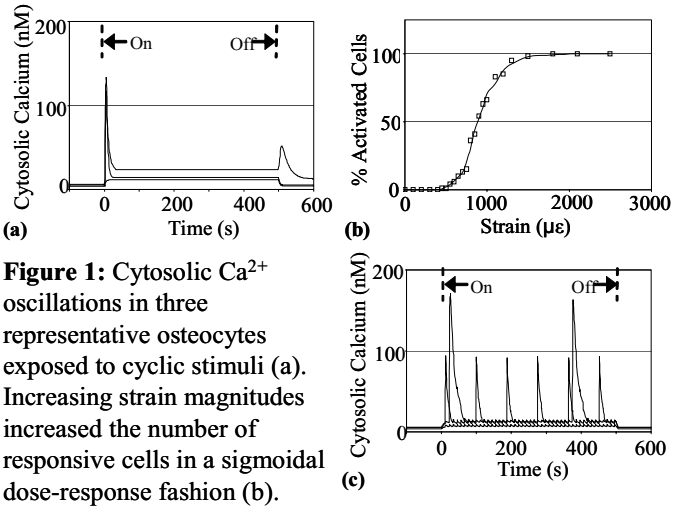

Figure 1: Cytosolic Ca^{2+} oscillations in three representative osteocytes exposed to cyclic stimuli (a). Increasing strain magnitudes increased the number of responsive cells in a sigmoidal dose-response fashion (b). Rest-inserted stimuli induced multiple high-magnitude Ca^{2+} transients in osteocytes (c).

In our agent based model of Ca^{2+} ion balance, distinct Ca^{2+} 'fingerprints' were not explicitly 'hardwired' within the model. Instead, they emerge via interactions between real cellular attributes (e.g., Ca^{2+} influx via stretch activated channels, ER Ca^{2+} stores). In our view, such an emergence based approach substantially extends model robustness and utility. Importantly, all Ca^{2+} response characteristics observed here (heterogeneous cell response, sigmoidal dose-response relations and multiple transients elicited by rest-inserted stimuli) were consistent with previous *in vitro* studies[3,5,6].

More broadly, it is possible to determine adaptation induced by mechanical waveforms at a variety of levels *in vivo* (from protein expression to cell proliferation and differentiation). Our model now provides a framework to functionally relate these adaptive events with unique Ca^{2+} fingerprints elicited *during* brief bouts of mechanical stimuli[2]. As a result, it may be possible to decipher the mechanisms underlying the distinct adaptive outcomes elicited by specific mechanical stimuli (e.g., why rest-inserted stimuli is dramatically osteogenic, but cyclic stimuli at identical loading magnitudes is not)[7]. Ultimately, the model and its expansions could serve as a powerful tool for optimization of bone tissue adaptation by enabling tailored design of loading waveforms that enhance cellular responses on the order of seconds.

REFERENCES

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