MECHANOSENSITIVITY OF BONE CELLS TO OSCILLATING FLUID FLOW INDUCED SHEAR STRESS MAY BE MODULATED BY CHEMO TRANSPORT

*Haut, T; *Donahue, H (A-NIH); *Haut-Donahue, T; *Yellowley, C (A-NIH); **Jacobs, C (A-NIH)
*Musculoskeletal Research Laboratory, Department of Orthopaedics and Rehabilitation, Penn State University, Hershey, PA 17033. +**Mechanical Engineering Department, Stanford University, Stanford, CA 94305. 650-736-0802, Fax: 650-725-1587, christopher.jacobs@stanford.edu

Introduction: It has been demonstrated that bone adapts to its physical loading environment and when customary bone loading is reduced, bone mass is removed resulting in osteopenia. However, the signal transduction pathways by which mechanical loading effects cellular responses in bone is unknown. Several possible biophysical signals believed to be involved in these signaling pathways include mechanical stretch, streaming potentials, chemotransport and lacunar-canalicular fluid flow. Recently, fluid flow has been shown to induce a variety of physiological responses such as increases in cytosolic calcium (Ca\(^{2+}\)) mobilization and prostaglandin E\(_2\) (PGE\(_2\)) release in bone cells (1,2). These studies though did not apply oscillating fluid flow (OFF) which is believed to be most representative of physiological conditions. Moreover, there have been no studies that have examined the effects of oscillating fluid flow induced shear stress and how this signal can be modulated by chemotransport. That is, how the transport of nutrients to the cells or possibly the removal of waste from the cells can alter the response to the fluid flow induced shear stress. Therefore, the goal of this study was to demonstrate that OFF induced shear stress is an important physical signal for bone cells that causes changes in Ca\(^{2+}\) mobilization and PGE\(_2\) release and that chemotransport can modulate these changes.

Methods: Cell Culture: The mouse osteoblastic cell line MC3T3-E1 was cultured in MEM-α supplemented with 10% fetal bovine serum and 1% penicillin/streptomycin and maintained at 5% CO\(_2\) at 37°C. Cell Culture: The mouse osteoblastic cell line MC3T3-E1 was cultured in MEM-α supplemented with 10% fetal bovine serum and 1% penicillin/streptomycin and maintained at 5% CO\(_2\) at 37°C.

PGE\(_2\) Assays: Preconfluent cells cultured on glass slides were mounted on a parallel plate flow chamber connected to a materials testing machine which generated oscillating fluid flow at 1 Hz. Cells were exposed to flow for 1 hour using three flow regimes, all producing a peak shear stress (PSS) of 20 dynes/cm\(^2\) accomplished by altering flow rate and increasing fluid viscosity with neutral dextran: 1) constant chemotransport (flow rate) of 4.5 ml/min, increasing the PSS from 5 dynes/cm\(^2\) to 20 dynes/cm\(^2\) with nutrient free HBSS. Image analysis software was used to capture and convert fluorescent signals into Ca\(^{2+}\) i concentration values. Ca\(^{2+}\) i transients of 50 nM or greater were considered responses.

Results: We found that oscillating fluid flow produced an increase in PGE\(_2\) production and elicited an increase in Ca\(^{2+}\) i mobilization in MC3T3-E1 cells. However, these responses were also found to be modulated by chemotransport. Oscillating fluid flow at 20 dynes/cm\(^2\) produced an increase in PGE\(_2\) release. However, at a constant PSS of 20 dynes/cm\(^2\), decreasing chemotransport (flow rates) from 43 to 28 to 18 ml/min significantly decreased PGE\(_2\) production from 34 ± 3.1±(SEM) to 25.8 ± 6.8 to 9.1 ± 1.8 pg/µg of total protein, respectively (p<.0001) (figure 1). In order to examine the effect of various amounts of neutral dextran on the cells, experiments were conducted in which slides of cells were placed in tissue culture dishes for 2 hours (to simulate 1 hour of flow and 1 hour of incubation performed in a standard experiment) with or without dextran and the media was analyzed for PGE\(_2\) production. There were no significant differences in PGE\(_2\) production between cells exposed to standard media or those cells exposed to 1g/100ml or 2g/100ml dextran. Calcium imaging was performed for 28 individual experiments (slides) and a total of 1398 cells. At a constant PSS of 20 dynes/cm\(^2\) reducing chemotransport (flow rate) from 18 ml/min to 11.5 ml/min significantly decreased the percent of cells responding with an increase in Ca\(^{2+}\) i from 87.6 ± 1.8% ±(SEP) to 75.2 ± 2.3 % (p<.01). At constant chemotransport (flow rate) of 4.5 ml/min, increasing the PSS from 5 dynes/cm\(^2\) to 8.7 dynes/cm\(^2\) of media increased the percent of cells responding from 13.5 ± 3.2 % to 32.6 ± 3.4 % (p<.01). Finally, in nutrient free HBSS only 10.7 ± 2.1 % of cells responded at 20 dynes/cm\(^2\) and was not significantly changed at 40 dynes/cm\(^2\), 8.5 ± 2.0 % (figure 2).

Discussion: It was demonstrated that intracellular calcium mobilization is increased with increased PSS while maintaining constant chemotransport (flow rate) suggesting that shear stress alone is an important physical signal that bone cells sense. However we have also demonstrated that both intracellular calcium mobilization and PGE\(_2\) production decrease with reduced chemotransport and constant PSS and that there was a marked decrease in Ca\(^{2+}\) i mobilization when nutrient free HBSS was used as the perfusing media. These data suggest that chemotransport is an important factor that may modulate the response of bone cells to OFF induced shear stress. The fact that the cellular responses to OFF induced shear stress are modulated by chemotransport is especially relevant for bone cells since they exist at some distance from the vasculature and therefore rely on loading-induced fluid flow to provide adequate nutrients and remove waste products.


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