

CELLULAR MECHANOTRANSDUCTION AND ITS MODULATION: AN ATOMIC FORCE MICROSCOPY STUDY.

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Atomic Force Microscopy (AFM) was used to apply forces onto cells with nanoscale precision and to estimate their material properties and resultant cellular strains. Bone is a material that adapts to mechanical usage, and osteoblasts detect strains experienced by the skeleton. We used AFM (ThermoMicroscopes Explorer) to apply forces and monitor increased intracellular calcium levels, an early event following a mechanical stimulus, in Fluo 3-labelled osteoblasts via an integrated Bio-Rad Radiance 2000 confocal microscope. Post hoc, the material properties and the strains applied were calculated. The cells responded to strains following a Boltzmann distribution (IC_{50} of $30,000\mu\epsilon$). The calcium signalling pathway(s) that were elicited in osteoblasts in response to strain were determined using pathway inhibitors and cytoskeleton disrupting compounds. Gadolinium ions blocked the calcium increases pointing towards the participation of mechanosensitive channels. Phospholipase C, intracellular calcium stores and ryanodine sensitive calcium stores were also involved. Responses were modulated by cytoskeleton disrupting drugs: cytochalasin B, jasplakinolide, nocodazole, taxol, acrylamide and diamide. In conclusion, AFM enables mechanotransduction and cellular mechanics to be examined at the single cell level. Force microscopy has been used herein to examine the behaviour of osteoblasts in response to strain, a process of key importance in understanding of the adaptation of the skeleton to mechanical forces.