The Receptor for Carboxyl-Terminal Parathyroid Hormone Regulates Osteocyte Cytoskeleton through Calcium Influx Dependent Mechanisms

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Parathyroid hormone (PTH), an 84-amino-acid polypeptide, is a major systemic regulator of calcium homeostasis and bone remodeling and elicits its classical functions by activating PTH/PTHrP receptors (PTHRs) on target cells. Carboxyl (C) fragments of PTH, secreted by the parathyroids in a calcium-dependent manner or generated by PTH proteolysis in the liver, circulate in blood at concentrations much higher than intact PTH(1-84) but cannot activate PTHRs. Receptors specific for C-PTH fragments (CPTHRs), distinct from PTHRs, are expressed by bone cells, especially osteocytes, in which they may regulate intercellular communication and cell survival.

Activation of CPTHRs previously was reported to modify intracellular calcium within chondrocytes. Our laboratory previously examined calcium signaling in several cell lines that express CPTHRs including osteocytes, ROS 17/2.8 osteosarcoma cells and RAW 264.7 myelomonocytic cells. Signaling studies demonstrated that CPTH induces voltage-sensitive calcium channel (VSCC) dependent calcium influx in the previously mentioned cells.

Since calcium is a major regulator of the cytoskeleton, we examined the effect of CPTHR-dependent calcium influx on cytoskeletal structure in the PTHR1-null osteocyte cell line (OC-59). OC-59 cells were treated with 100 nM CPTH (53-84) for 2 or 10 minutes and then examined by immuno-fluorescent staining of cytoskeletal components (actin and vinculin). OC-59 cells treated with 100 nM PTH(53-84) for 10 minutes demonstrated marked actin and vinculin condensations compared to cells treated with vehicles or cells treated with PTH(53-84) for only 2 minutes.

The specificity of the cytoskeletal changes in response to CPTH treatment was examined by treating cells with the mutant CPTH analog, [Ala^{55-57}]PTH(53-84), which does not bind to CPTHRs nor induce a calcium signal in OC-59 cells. [Ala^{55-57}]PTH(53-84) failed to induce any cytoskeletal changes in OC-59 cells treated for 10 minutes. Similar results were observed using PTH 1-34, which does not bind to or activate CPTHRs.

The role of calcium influx in cytoskeletal changes induced by CPTH treatment was examined by blocking calcium influx using gadolinium chloride (GdCl). PTH(53-84) failed to induce cytoskeletal changes in OC-59 cells pretreated with GdCl (1 and 10 mM). In our previous calcium studies, GdCl completely inhibited the calcium signal in response to CPTH treatment. Collectively these data suggested that calcium signals induced by CPTHRs may play an important role in regulation of osteocyte cytoskeletal assembly and structure.