Role of Voltage Dependent Calcium Channels in Carboxyl-Terminal Parathyroid Hormone Receptor Signaling

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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.

To further investigate the mechanism of action of CPTHRs in osteocytes, cytosolic free calcium, (Ca_i^{++}) was measured in the PTHR-null osteocytic cell line OC 59, which expresses abundant CPTHRs but no PTHRs. Ca_i^{++} was assessed by single-cell ratiometric microfluorimetry in Fura -2 loaded OC 59 cells that were pretreated for 16 h with 0.3 mM 8Br-cAMP, which increases CPTHR expression in these cells A rapid and transient increase in Ca_i^{++} was observed in OC59 cells in response to the CPTH fragment hPTH(53-84) (250 nM). Similar results were obtained using analogs of longer CPTH fragments, such as hPTH(13-84) and hPTH(23-84). No Ca_i^{++} signal was observed in COS-7 cells, in which CPTHR binding also cannot be detected. A mutant CPTH analog, [Ala^{55-57}]hPTH(53-84), which does not to bind to CPTHRs, also failed to elicit an increase in Ca_i^{++} in OC59 cells, as did hPTH(1-34).

The Ca_i^{++} response to hPTH(53-84) required the presence of extracellular calcium and was blocked by inhibitors of voltage-dependent calcium channels (VDCC), including nifedipine (100 nM), w-agatoxin IVA (10 nM) and w-conotoxin GVIA (100 nM). Interestingly, VDCC inhibitors also decreased specific binding of the radioligand ^{125}I-[Tyr^{34}]hPTH(19-84) to CPTHRs on OC59 cells.

We conclude that activation of CPTHRs in OC59 osteocytic cells leads to a rapid increase in influx of extracellular calcium, most likely through opening of VDCCs. VDCCs could interact directly with CPTH ligands or with CPTHRs. Alternatively, they may be required to maintain Ca_i^{++} levels necessary for CPTHR binding to extracellular CPTH ligands. Calcium influx through VDCCs may play a critical role in CPTH receptor signaling in bone cells.