Osteocytes are the most abundant cells in bone, yet their functions are still poorly understood. Osteocytes express several membrane receptors, including the parathyroid hormone (PTH) type 1 receptor (PTH1R)\(^1\), estrogen receptors (both \(\alpha\) and \(\beta\)\(^2-5\)), and, as we recently have reported, a novel receptor that specifically recognizes the carboxyl-terminal region of PTH, the carboxyl-terminal PTH receptor (CPTHR)\(^6,7\).

PTH is a single-chain polypeptide comprised of 84 amino acids and its main function is to maintain constant the serum calcium concentration. PTH accomplishes this via activation of a G-protein coupled receptor, the type 1 PTH/PTH-related peptide receptor (PTH1R), mainly expressed in kidney\(^8\).

The amino-acid sequence of PTH is highly conserved among species and this high degree of evolutionary conservation strongly suggests the possibility of additional, independent biological function(s) for the C-terminal region of the PTH molecule. Indeed, evidence of cellular receptors with specificity for the C-terminal portion of PTH(1-84) ("CPTHRs") has accumulated steadily over the past 25 years, as recently reviewed\(^9\). We reported abundant expression of CPTHRs (2-3 x 10\(^6\)/cell), detected using \(^{125}\)I-[Tyr\(^{34}\)]hPTH(19-84) as radioligand, on the surface of clonal osteocytic cells ("OC cells") isolated from calvarial bone of fetal PTH1R-null mice, thus providing the first conclusive evidence that CPTHRs exist independently of PTH1Rs\(^6,10,11\).

Recently we have identified specific structural determinants of CPTHR binding\(^7\) and we demonstrated that CPTHR activation in osteocytes leads to increased cell death, an effect opposite to that reported for the PTH1R, and to an increase in cell-to-cell communication.

The author has no conflict of interest.

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Accepted 31 July 2005
morpheometric parameters. This effect of PTH was specifically inhibited by verapamil, a VOCC blocker. Lastly, Jilka et al. reported that PTH exerts its anabolic effect on bone by suppressing osteoblast and osteocyte apoptosis. They reported that the intermittent versus sustained effect of PTH is related to proteosomal degradation of Runx-2. Interestingly we have demonstrated that CPTH activation on osteocytes exerts a pro-apoptotic effect, an action opposite that of PTH1R activation.

**CPTH and osteocytes**

The high levels of CPTH expression by OC cells enabled a reliable analysis, using OC59 cells and the CPTH radioligand $^{125}$I-[$^{15}$Tyr$^{54}$]-hPTH(19-84) (which does not bind to the PTH1R), of the structural determinants for ligand binding. As recently reported, N-terminally truncated human PTH peptides hPTH(7-84), [Tyr$^{34}$]hPTH(11-84), [Tyr$^{34}$]hPTH(13-84), [Tyr$^{34}$]hPTH(19-84) and [Tyr$^{34}$]hPTH(24-84) displaced the radioligand as effectively as hPTH(1-84) (IC$_{50}$: 10-40 nM), whereas a group of shorter peptides, including hPTH(28-84), hPTH(34-84), hPTH(37-84), [Asn$^{76}$]hPTH(39-84) and hPTH(53-84), bound with significantly lower apparent affinity (IC$_{50}$: 200-600 nM). Further minimal N-terminal truncation beyond position 53, as in hPTH(55-84), hPTH(57-84) and hPTH(60-84), effectively abolished measurable binding affinity for CPTH-Rs (IC$_{50}$ >> 10,000 nM) highlighting the presence of at least two domains required for maximal binding affinity – one within the sequence hPTH(24-27), (“binding domain 1; BD1”) and another represented by the dibasic sequence (Lys$^{55}$-Lys$^{58}$), termed “binding domain 2 (“BD2”). Further analysis of the intact hormone pointed to the presence of additional major determinants of binding affinity within the region hPTH(55-84), thereby defining a third “binding domain” (“BD3”). To identify key residues involved in the contribution of BD3 to overall CPTH ligand binding affinity, clustered triple-alanine substitutions were introduced across the sequence of hPTH(53-84), to produce nine mutant hPTH(53-84) peptides. Three of these peptides, with substitutions at positions 71-74 (“M9”), 64-66 (“M6”) and 55-57 (“M7”), respectively, showed dramatic (roughly 100-fold) reductions in apparent affinity. Further analysis of additional peptides harboring single-alanine substitutions within these regions identified three key residues - Asn$^{77}$, Lys$^{65}$ and Lys$^{72}$ - that appear to be critical for high affinity binding to CPTH-Rs.

As osteocytes are terminally differentiated osteoblasts, it was of interest to determine if CPTH activation might play a role in regulating apoptosis in the OC cells. We found that incubation of OC cells, which lack functional PTH1R genes, for 6 hours with 100 nM hPTH(1-84) led to increased nuclear pyknosis and chromatin condensation, as revealed by DNA staining with Hoechst dye 33258. Increased apoptosis also was observed in response to the intact hormone or the short fragment hPTH(53-84) using a TUNEL immunocytochemical assay.

Our initial signaling studies demonstrated that CPTH activation induces a rapid influx of calcium from the extracellular compartment, likely via opening of calcium channels. Since calcium is a major regulator of the cytoskeleton, we examined the effect of CPTH-dependent calcium influx on cytoskeletal rearrangement. OC59 cells were treated with 100nM hPTH(53-84) for 2 and 10 minutes and then were examined by immuno-fluorescent staining of cytoskeletal components (vinculin and actin). OC59 cells treated with the CPTH fragment demonstrated a marked actin and vinculin condensation, suggestive of a rapid modification of the cytoskeleton.

The specificity of this effect was examined by treating the cells with the mutant peptide [Ala$^{55-57}$]hPTH(53-84), which does not bind or activate calcium influx and, as expected, the mutant analog failed to induce any cytoskeletal changes in OC cells. The role of calcium influx was examined by blocking calcium influx with gadolinium chloride (GdCl) (1 and 10 μM). When OC59 cells were treated with GdCl, hPTH(53-84) failed to induce cytoskeletal changes, indicating that calcium influx might play an important role in the regulation of osteocyte cytoskeletal assembly and structure.

Thus, in summary, it seems possible that PTH might regulate osteocyte function via at least two receptor systems, the PTH1R and the CPTH, although many questions still remain.

Frost had proposed that systemic hormones might alter bone remodeling by changing the thresholds at which mechanosensory cells in bone respond to differing intensities of mechanical stress or loading. It therefore will be important, in the future, to further define the effects of PTH, acting both via the PTH1R and the CPTH, on osteocytes undergoing mechanical stimulation.

**References**


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