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Presentation Number:	M451
Title:	Receptors for Carboxyl-PTH on Pheochromocytoma Cells Trigger Calcium Influx and Regulate Secretion
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Parathyroid hormone (PTH) is a major regulator of calcium homeostasis and elicits its functions by activating PTH/PTHrP receptors (PTHRs) on target cells. Carboxyl (C) fragments of PTH, secreted by the parathyroids or generated by PTH proteolysis in the liver, circulate in blood at concentrations 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. Activation of CPTHRs has been 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 CPTHR activation induces an increase in intracellular calcium that is dependent on voltage-sensitive calcium channels (VSCC).
Since it is known that calcium influx through VSCCs commonly regulates the secretion process in various cell types, we investigated CPTHR expression and function(s) in pheochromocytoma-derived PC-12 cells. These cells express various types of VSCCs, secrete norepinephrine (NE) and maintain a neuroendocrine phenotype in culture. To determine if PC-12 cells express CPTHRs, cells were incubated with biotinylated hPTH(23-84) preincubated with fluorescent streptavidin, in the presence or absence of excess non-biotinylated CPTH peptide, and analyzed by fluorescent-cell scanning. Specific binding was reflected by a shift from 95% to 45% of cell-associated fluorescence exceeding a predefined threshold. Upon stimulation of fura-2-loaded PC-12 cells with 100 nM hPTH(23-84), a rapid and transient increase in cytosolic calcium was observed. This response was completely abolished by pre-treatment with 10 mM gadolinium chloride (GdCl). PC-12 treatment with hPTH (23-84) (100 nM) for 60 minutes induced NE release (three fold compared to control). A smaller response was observed after 30 minutes and none was seen at 10 minutes. No NE release was observed in PC-12 cells treated for 60 minutes with 100 nM hPTH(1-34) or with a mutant CPTH peptide ([Ala 55-57]hPTH(53-84)), neither of which bind to the CPTHR. To examine the role of calcium influx in mediating CPTH-induced NE release, PC-12 cells were pre-treated with 10 mM GdCl for 10 minutes before stimulation with 100 nM hPTH(23-84). GdCl completely abolished the effect of PTH(23-84), suggesting that CPTHRs expressed in PC-12 cells can regulate their secretory functions by calcium-dependent mechanisms. These observations indicate that CPTHRs are expressed in cells outside the bone tissue and may mediate extra-skeletal actions of CPTH fragments.