Signaling and Expression of a Truncated, Constitutively Active Human Insulin Receptor

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Background: Insulin signaling is indispensable for key metabolic pathways in the periphery. Several studies have demonstrated that insulin signaling is also important for brain function. Early stage clinical trials report a positive impact of intranasal insulin on memory recall in young subjects and patients with mild cognitive decline or Alzheimer's disease (AD). However, the underlying molecular mechanisms involved are not well understood.

Purpose: Here we sought to investigate the role of insulin in neuronal physiology by overexpressing a constitutively active human insulin receptor (Lebwohl et al., 1991) in rat pheochromocytoma (PC12) and primary hippocampal neurons.

Methods: Cells were transfected with either pCI-ires-dsRed, a mammalian expression plasmid encoding a red fluorescence protein (ds-Red), or pCI-HA-IRβ-ires-ds-red, the construct with a truncated human insulin receptor beta subunit (IRβ), via either electroporation (PC12 cells) or a targeted lentiviral delivery system (neurons). The expression of IRβ receptor in PC12 cells was corroborated by the expression of the red fluorescent protein. Photomicrographs of mixed primary hippocampal cultures confirmed expression of the lentiviral plasmid in neurons. The expression level and effect of IRβ overexpression on insulin signaling was confirmed in PC12 cells by performing immunoblots using antibody against HA-tagged IRβ and measuring pAkt/Akt ratio.

Results/Conclusions: Our data show that overexpression of insulin receptor enhances neurite outgrowth and increases the pAkt/Akt ratio in PC12 cells. Overexpression of truncated receptor increased insulin signaling compared to control. This initial characterization provides insights into future intervention approaches to combat reduced insulin signaling in AD and/or aging.