Signaling and expression of a truncated, constitutively active human insulin receptor in hippocampal neurons

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Objectives:
Insulin signaling is indispensable in the periphery and it is becoming clear that insulin is also important for normal 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). To address alternative strategies for enhancing insulin signaling in the brain, we have conducted a series of experiments using a constitutively active human insulin receptor (IR). Distribution and functional characteristics were evaluated using rat primary mixed hippocampal cultures.

Methods/Results:
Cells were transfected with either a mammalian expression plasmid encoding a red fluorescence protein (ires-dsRed), or a construct containing the truncated human IR beta subunit (HA-IRβ-ires-dsRed) via a targeted lentiviral delivery system. The expression of IRβ receptor in hippocampal cultures was corroborated by the expression of the red fluorescent protein. A silencing site (RE1) was used to restrict expression to neurons. Photomicrographs of mixed primary hippocampal cultures confirmed expression of the lentiviral plasmid in neurons and astrocytes. The expression level and effect of IRβ overexpression on insulin signaling was confirmed by performing Western immunoblots measuring pAkt/Akt ratio and immunocytochemistry assays with antibody against HA-tagged IRβ. Barium currents were recorded in cultures infected with lentivirus using whole-cell patch-clamp electrophysiology and results were quantified at three different time points.

Conclusions:
Western blots of infected mixed hippocampal cultures provide evidence that transfection with the truncated IRβ plasmid confers a greater response to insulin treatment. Lentiviral infection of mixed primary hippocampal cultures was successful for all our constructs suggesting that this approach is viable for enhancing insulin signaling. Patch-clamp recordings do not currently show a significant difference between the two groups at all three time points. This initial characterization provides insights into future intervention approaches to combat cognitive decline in AD and/or aging using molecular methods to enhance insulin signaling.