## Acute insulin treatment of hippocampal neurons highlights new mechanisms of action

Shaniya Maimaiti[1] • Hilaree Frazier, MS[1] • Katie Anderson[1] • Lawrence Brewer, PhD[1] • Nada Porter, PhD[1] • Olivier Thibault, PhD[2] • Olivier Thibault, PhD[1]

*[1]Pharmacology and Nutritional Sciences, University of Kentucky • [2]Pharmacology and Nutritional Sciences*

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Our lab studies the effects of different insulin formulations, zinc-containing insulin and zinc–free insulin, on cognition. We identified that intranasal delivery of Levemir or Humalog (zinc-containing insulins) improve cognition in the aged F344 rats (Maimaiti et al., 2015). On the other hand, intranasal Apidra® (zinc-free insulin) failed to improve cognition in the aged F344 rats (Anderson et al., 2016). Growing evidence supports the concept that brain insulin defects in signaling or receptor numbers are associated with memory decline in Alzheimer's disease (AD) and advanced age.

Although intranasal insulin enhances memory in AD patients, it also does so in young adults subjects with normal insulin signaling at baseline. While prior work has provided evidence that ion channels (AMPA, NMDA, GABA), glucose transporter-4, insulin degrading enzyme and vascular function, very little work has focused on voltage-gated calcium channels and intracellular Ca2+ as a target of insulin action in the brain. Prior work also shows intracellular Ca2+ is a key neuronal molecular regulator of hippocampal-dependent memory. Elevated intracellular Ca2+ levels in hippocampal neurons have been shown in aged animals with poor spatial memory. We have shown that insulin reduces the Ca2+ -dependent afterhyperpolarization (AHP) in hippocampal neurons in both young and aged animals (Maimaiti et al., 2015). However, the underlying mechanism that underlies this reduction has not been studied in depth.

The goal of the present work is to test the hypothesis that insulin improves memory by reducing Ca2+ dysregulation. We used whole cell patch clamping and Ca2+ imaging techniques to measure voltage-gated Ca2+ currents (VGCCs), intracellular Ca2+ levels from 13-17 DIV primary hippocampal neurons in culture. Active Apidra (10nM, rapid-acting, zinc-free insulin), boiled Apidra, reconstituted human insulin, and zinc were tested acutely for effects on VGCCs. Results show that both 10 nM Apidra and reconstituted insulin reduces Ca2+ current. 10 nM Apidra did not reduce resting Ca2+ levels or spontaneous Ca2+ transient, but did reduce KCl-induced intracellular Ca2+ transients. These effects were attenuated by pretreatment with an insulin antibody. Together, these results indicate insulin can reduce intracellular calcium levels during neuronal depolarization, highlighting a potential mechanism for reducing the AHP and improving memory.

## Indentification of Novel Tau Interactions with Endoplasmic Reticulum Proteins in Alzheimer's Disease Brains

Grant Nation[1] • Shelby Meier[1] • Danielle Lyons, PhD[1] • Alexandria Ingram[1] • Jing Chen[2] • John Gensel, PhD[1] • Haining Zhu, PhD[2] • Peter Nelson, MD, PhD[3] • Jose Abisambra, PhD[1]

*[1]Physiology, University of Kentucky • [2]Molecular and Cellular Biology, University of Kentucky • [3]Pathology, University of Kentucky*

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Alzheimer's disease (AD) is a progressive neurodegenerative disorder pathologically characterized by the formation of extracellular amyloid plaques and intraneuronal tau tangles. Neurofibrilary tangles (NFTs) are composed of aberrant aggregates of the tau protein. Tau in NFTs is abnormally folded, hyperphosphorylated, oligiomerized, and mislocated. In AD, tau becomes neurotoxic however the mechanism of this toxicity has not been identified. A possible key mechanism in neurodegeneration is impaired protein synthesis. This study seeks to find tau-associated ER proteins and, in particular, those connected to RNA translation.