Isoform-Specific Inactivation and Aggregation of CaMKII under Ischemic-Like Conditions

Ross Nelson [1]; Andy Hudmon, Ph.D. [2,3]; Derrick Johnson [2]; Swarna Ramaswamy, Ph.D. [3,] and Aarti Chawla [2,3].

[1]Indiana University-Purdue University IPREP Program, [2]Department of Biochemistry and Molecular Biology, Indiana University School of Medicine, [3]Stark Neuroscience Research Institute, Indiana University School of Medicine, Indianapolis, IN 46202

Indiana University-Purdue University Indianapolis

Calcium-Calmodulin Dependent Protein Kinase II (CaMKII), an enzyme critical for learning and memory, inactivates and self-associates into sedimentable aggregates following ischemic insults such as stroke or traumatic brain injury; the extent of inactivation correlates increased neuronal dysfunction and death. CaMKII α and β—isoforms found primarily in neurons—are well documented in their response to ischemic stress; α aggregates and undergoes catalytic inactivation quickly while β does not. However, γ and δ—primarily found in glial cells—are not well studied under these conditions. Previous research by our lab suggests that loss of CaMKII signaling in astrocytes may contribute to reduced glutamate uptake and neurotoxic ATP release. Therefore, there is a need to elucidate the role of the astrocytic CaMKII isoforms in ischemic stress. This study aims to investigate CaMKII δ and γ's response to artificial ischemic conditions compared to CaMKII α. Activity assay of cell lysates expressing the four different human genes of CaMKII (α, β, γ, and δ) reveal that, under artificial ischemic conditions, δ undergoes very minimal loss of activity over time while γ experiences robust inactivation. We then used light scattering to compare α, δ, and γ sedimentation in real time and found that δ had an aggregation profile similar to α yet γ's was radically different. A follow-up time-course sedimentation assay suggests that δ becomes sedimentable and undergoes an upwards molecular weight shift akin to α over time, indicative of autophosphorylation, but that γ begins partially sedimentable before becoming completely soluble upon activation, contrary to our hypothesis. This suggests that each isoform responds differentially to activation under ischemic-like conditions and that aggregation is not necessarily correlative with inactivation. We are currently characterizing endogenous astrocytic CaMKII expression and activity to later determine if these findings persist in a cellular environment under ischemic-like conditions.

Mentor: Andy Hudmon, Stark Neurosciences Research Institute, IU School of Medicine, IUPUI, Indianapolis, IN