

Differential Effects of Amyloid-Beta Treatment on both Mitochondrial Function and Calcium Homeostasis at Nerve Terminals and Glia

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There is strong evidence that in Alzheimer's Disease (AD), synapses are impaired before neurons degenerate and senile plaques accumulate (1). This squarely places the synapse as a target in the early pathogenesis of AD. Because synapses exist in a dynamic microenvironment with non-neuronal cells, glial and endothelial cells have been implicated as targets and participants in the progression of AD (2,3). For example, A β treatment has been shown to alter the ability of astrocytes to ameliorate neuronal oxidative insults (4-6) and to have specific effects on mitochondrial respiration and cellular Ca²⁺ balance (5,7). However, the A β -mediated derailment of the functional and cellular interactions among the neurons, glia, and capillaries are insufficiently understood (8).

We are testing the hypothesis that one of the earliest A β -mediated neurotoxic events may occur primarily in the mitochondria of perisynaptic glia, altering glial cell Ca²⁺ homeostasis (5,9). In this scenario, A β subsequently induces neurodegeneration by predisposing nerve terminals to activity-induced damage following reduction of the supportive/protective capacity of the glia that sheath the nerve terminal, or perhaps by exposure to substances secreted from the microvascular endothelium. Most studies that have addressed mechanisms of A β -induced toxicity have used primary cell culture models or relatively complex model systems comprised of multiple cell types. We have taken a different approach to study the initiation and progression of the earliest events of A β -induced neurotoxicity on intracellular and intercellular function. We have developed organotypic slice and pure nerve terminal or glial cell culture preparations of the rat posterior pituitary (PP), and are using this simplified model system in combination with cellular imaging methods employing Ca²⁺-sensitive or membrane potential-sensitive dyes that selectively localize to specific sub-cellular compartments. The PP preparation has the advantage in that it is comprised almost solely of brain nerve terminals (without axons or cell bodies), a network of a single type of astrocyte (pituicyte), and a rich microvasculature.

We used confocal methods to probe the morphological characteristics of the main players in this model system, and have begun to characterize functional aspects of the interaction between these components. The energy states of single or populations of individual mitochondria in nerve terminals or pituicytes were assessed by digital fluorescence imaging or high-throughput fluorescence plate reader and JC-1, a fluorescent ratiometric indicator of mitochondrial inner membrane potential. Similar results were obtained using the mitochondrial selective potentiometric dye TMRM. Following 24 hr treatment with 25 μ M A β 25-35, isolated pituicytes exhibited on average ~70% decrease in ratio values compared to vehicle- or reverse peptide-treated control values. The level of mitochondrial inhibition after 24 hr treatment was comparable to that induced by treatment with the 20 μ M FCCP, a protonophore that uncouples mitochondria. Surprisingly, treatment of isolated nerve terminals with A β did not produce a significant decrease in ratio values compared to control. Likewise, activity induced changes in mitochondrial Ca²⁺ import, as measured by the mitochondria-selective Ca²⁺ dyes Rhod-2, -FF and -5N, were decreased after A β treatment in pituicytes but not in nerve terminals. Consistent with the differential effects of A β on mitochondrial

inner membrane potential and mitochondrial Ca^{2+} dynamics, 24 hr A β -treated pituicytes exhibited impaired cytosolic Ca^{2+} homeostasis as compared to control cells. Treated and control nerve terminals responded to Ca^{2+} challenges similarly. Using fluorescently tagged A β peptides, we demonstrated that differences between pituicytes and terminals in the uptake or trafficking of exogenous A β might in part underlie the observed sensitivity of pituicytes to A β

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