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A β toxicity to SHSY-5Y human neuroblastoma cells

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Alzheimer's disease (AD) is a progressive neurodegenerative disorder that results in the loss of memory, language deterioration, confusion, restlessness, and eventually destroyed cognition. It is characterized by an accumulation of beta amyloid (A β) plaques and tangles in the brain. Increased oxidative stress has been regarded as an early event underlying the progression of AD and there is evidence that A β can cause oxidative stress. In our lab, we are using SHSY-5Y human neuroblastoma cells (SH cells) as a model to look at NADPH-oxidase, an enzyme that may be involved A β induced toxicity to the cells. NADPH-oxidase is a multi-subunit enzyme that catalyzes molecular oxygen to form reactive oxygen species (ROS). In order for this enzyme to be activated, its cytosolic components must translocate to the membrane. One of its cytosolic components is p47-phox. We stimulate cells with oxidative agents such as menadione and H₂O₂ to monitor the translocation of p47-phox to the membrane. Because the production of ROS may be one of the factors leading to neuronal death, we chose to look at the activation of NADPH-oxidase. SH cells were treated with menadione (stimulator), H₂O₂ (stimulator) and DPI (inhibitor) for different time intervals. After treatments, the cells were harvested and treated with a lysis buffer to lyse the cells. After the cells were lysed, we put them through ultra-centrifugation and collected the membrane and cytosolic fractions. Western Blots were performed on both the membrane and the cytosolic fractions to look for the presence of p47-phox. From the Western Blot we saw that there was an increase in the p47-phox levels in the membrane fraction after exposing the cells to menadione and H₂O₂. Our results show that treating the cells with oxidative agents caused an increase in the p47-phox level in the membrane fraction thus leading to the activation of NADPH-oxidase.

