Study shows Alzheimer's disease-related peptides form toxic calcium channels in the plasma membrane

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Alzheimer's disease is triggered by the inappropriate processing of amyloid precursor protein to generate excess amounts of short peptide fragments called A-beta. For many years, the neurodegeneration associated with Alzheimer's disease was thought to be caused by the buildup of A-beta in insoluble, fibrous plaques. However, increasing suspicion now falls on smaller, soluble A-beta complexes as the toxic form of the protein, partly through their ability to induce excess calcium influx into cells, which disrupts synaptic signaling and stimulates cell death. A new study in The *Journal of Cell Biology* uses high-resolution imaging to reveal that A-beta oligomers elevate calcium by forming calcium-permeable pores in the plasma membrane.

A-beta oligomers could induce calcium influx by physically disrupting the cell's outer membrane or by activating endogenous calcium channels. But studies have also shown that A-beta peptides can form calcium-permeable pores themselves in both artificial and cell membranes. A limitation of experimental techniques used to date, says Angelo Demuro, from the University of California, Irvine, is that they only monitor the activity of one or two channels at a time. In addition, different groups have obtained disparate results regarding the properties of A-beta channels using this approach.

To overcome these problems, Demuro and colleagues developed an alternative method to measure the activity of calcium channels in living

cells. "We can simultaneously record the behavior of thousands of channels using an imaging technique we call optical patch-clamping," Demuro explains. In this approach, frog eggs are filled with a calcium-sensitive dye, and the researchers observe the part of the cell nearest to the cell's outer membrane. When membrane channels open to let calcium into the cell, small fluorescent flashes indicate the duration and extent of calcium influx at each individual pore.

Demuro et al. found that, just twenty minutes after A-beta oligomers were added to the eggs, they displayed flickering spots of fluorescence signifying calcium influx through single membrane channels. This influx was unlikely to be through endogenous channels activated by A-beta because frog eggs barely express calcium channels of their own. Moreover, A-beta aggregates weren't simply disrupting the eggs' membrane, as the influx was inhibited by zinc ions, which block calcium-permeable pores.

A-beta oligomers therefore form calcium-permeable channels of their own in the membrane. Demuro and colleagues characterized the properties of these pores by simultaneously imaging the activity of thousands of channels in a single membrane region. "They are all different," says Demuro. "[The pores] show a wide variety of behaviors." Most pores opened infrequently and only let in small amounts of calcium, but some opened more often and channeled large amounts of calcium into the cell. Though few in number, Demuro et al.'s measurements suggest that this latter type of pore may be largely responsible for the toxic increase in cytoplasmic calcium levels.

Differences in the properties of individual pores may be caused by differences in the number of A-beta peptides assembled into each channel, with higher-order oligomers forming the more active species of pore. "It would be nice to visualize how many A-beta peptides each pore has and whether this is related to the activity of the channel," Demuro

says. If pore activity is affected by the oligomerization state of A-beta, it appears that A-beta peptides continue to assemble after their insertion into membranes, as the pores became more active as eggs were exposed to A-beta oligomers for longer periods. This increase in calcium influx over time may be related to the gradual progression of Alzheimer's symptoms.

Beyond Alzheimer's disease, Demuro et al.'s approach may help explain the pathogenesis of other neurodegenerative disorders like Parkinson's and Huntington's disease, in which misfolded and aggregated proteins have also been reported to form calcium-permeable channels.

More information: Demuro, A., et al. 2011. *J. Cell Biol.* doi:10.1083/jcb.201104133

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