Familial Alzheimer’s Disease and the Disruption in Ca\(^{2+}\) Signaling

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Introduction:

Alzheimer is a fatal neurodegenerative disease. Familial Alzheimer’s disease (FAD) is caused by mutations in presinilins (PS) which are a family of transmembrane proteins. Mutations in presinilins lead to abnormalities in Ca\(^{2+}\) signaling in the cells. Inositol 1,4,5-trisphosphate is a Ca\(^{2+}\) release channel that loses its normal function in patients with AD. This experiment demonstrated that two PS mutants, PS1 (M146L) and PS2 (N141I), interact with (InsP3R) Ca\(^{2+}\) release channel and affect its gating activity. These interactions cause abnormal increase in Ca\(^{2+}\) signaling through facilitated release of Ca by endoplasmic reticulum which stimulates Amyloid Beta processing. Amyloid beta (Aβ) forms amyloid plaques in the brain of patients with Alzheimer’s disease. InsP3R-deficient cells were used to show that this exaggeration in Ca\(^{2+}\) signaling is involved in Aβ generation in PS mutants. Overall, the point of this experiment is to find a mechanism underlying altered Ca\(^{2+}\) signaling which happens in AD cells.
Experimental System:

- Sf9 cells were isolated from insects.
  - Reason: these cells express single isoform of InsP3R which has the gating and permeation characteristics very similar to those of mammalians.

- Recombinant DNA Technology was used.
  - Reason: to make recombinant Baculovirus to infect Sf9 cells.
  - Sf9 cells infected with Baculovirus -in which PS genes were inserted- express PS1 and PS2 mutated proteins.

- Western Blotting was used.
  - Reason: To confirm the expression of PS1 and PS2.

- Immunocytochemistry was also used.
  - Reason: To make sure if PS in Sf9 cells is localized.
• Single-Cell Ca²⁺ Imaging was used
  ➢ Reason: To measure the changes in concentration of Ca²⁺.

Experiments and Results:

• **Experiment 1**: The effect of PS1 expression on InsP3 channel
  ➢ Baculoviruses infected isolated nuclei from Sf9 cells so that these cells can express PS1 WT or M146L proteins. This infection was conducted in two different situations. In the first situation the solution of InsP3, as a ligand for its receptor, was not added. In the second situation the saturated amount of InsP3 was used.
  
  ➢ **Results**: PS1 alone cannot evoke the channel activity. However when a saturated solution of InsP3 was added, oscillations were observed in the current recording line. This experiment showed that InsP3 channel is activated when both PS1 and InsP3 are present.

• **Experiment 2**: The effect of PS2 on InsP3 channel activity in Sf9 cells. Baculoviruses infected isolated nuclei from Sf9 cells so that these cells can express PS2 WT or N141I proteins
  
  ➢ **Results**: Again high oscillations were observed in current recordings when a saturated solution of InsP3 was added.

• **Experiment 3**: In order to see if the observed effects of FAD mutant PS on the InsP3 channel is the reason for alteration in [Ca²⁺] signaling, Ca²⁺ signals in DT40 cells were recorded. Crosslinking the B cell receptor (BCR) evoked InsP3R-mediated Ca²⁺ signals. In control cells a high concentration of Anti IgM caused a considerable increase in amount of Ca²⁺ signaling.
  
  ➢ **Results**: In those cells that expressed PS1 WT the same magnitude of Ca²⁺ signaling response was observed. However in those cells that expressed PS1 M146L the exaggerated Ca²⁺ response was obvious. This exaggerated Ca²⁺
response in DT40 cell system was very similar to the response that is observed in FAD patients who express mutant PS.

Two hypotheses for the observed exaggeration of [Ca2+] responses are the increased expression of Ca2+ release channels and overfilling of ER Ca2+ stores. The levels of types 1 and 3 InsP3R were observed to be equal in all three cell lines (control, PS1 WT, and PS1 M146L.)

- **Experiment4:** In order to test the overfilling of ER Ca stores hypothesis, [Ca] responses were measured by the Ca2+ ionophore ionomycin. To make sure that the Ca2+ responses are entirely due to the Ca2+ release from intracellular compartments, ionomycin was applied in the absence of extracellular Ca2+.

  ➢ **Results:** In both PS1-M146L and PS1-WT cell, a rapid release of Ca2+ was caused by ionomycin. This response didn’t change in PS1-WT but it was decreased in the PS-1M146L expressing cells. The results demonstrated that ER Ca2+ stores aren’t overfilled in PS1-expressing cells.

- **Experiment5:** To test the changes in amount of Ca2+ in ER stores, Mag-Fura-2 was used as a low affinity fluorescence indicator. The Ca2+ leak rate of the ER was measured in all three cell lines with ER stores filled with Ca2+.

  ➢ **Results:** Control and PS1-WT cells showed a similar Ca2+ leak rate but a greater rate was observed in the PS1-M146L expressing cells. However after addition of heparin, which is an InsP3R inhibitor, this enhanced Ca2+ leak rate was eliminated. The results suggest that PS1-M146L protein changes the gating
activity of the InsP3R which results to the increased Ca2+ leakage from ER stores and therefore exaggerated Ca2+ signaling.

- **Experiment 6**: To find a relation between FAD PS1 expression and the altered InsP3R-mediated Ca2+ release in brain neurons. Ca2+ permeability of the ER was measured in cortical neurons that were isolated from E15-E16 mouse brains.

  - **Results**: In control or PS1-WT expressing cells, the addition of 33 nM solution of InsP3 could not evoke Ca2+ release from ER, but Ca2+ release response was observed in PS1-M146L cells. Similar to the result of experiments with non-neuronal cells, InsP3R is sensitized and exaggerated Ca2+ release occurs in FAD mutant PS1 neurons.

- **Experiment 7**: To examine the role of InsP3R in APP processing, APP and PS1-expressing cells lacking InsP3R (KO) were generated.

  - **Results**: The enhanced Aβ secretion that was observed in mutant PS1 cells, were reduced. The results demonstrate that altered APP processing which occurs in mutant PS1-M146L is dependent on the InsP3R.

**Conclusion:**

- Mutations in presinilins lead to abnormalities in Ca²⁺ signaling in the cells.
- InsP3 (a Ca2+ release channel) loses its normal function in patients with AD.
• PS mutants, PS1 (M146L) and PS2 (N141I), interact with (InsP3R) Ca2+ release channel and affect its gating activity.
• These interactions cause abnormal increase in Ca2+ signaling through facilitated release of Ca by endoplasmic reticulum which stimulates Amyloid Beta processing.

**Significance:**

Alzheimer is a fatal neurodegenerative disorder. As it was mentioned before, Familial Alzheimer’s disease (FAD) is caused by mutations in presinilins (PS) which are a family of transmembrane proteins. Mutations in presinilins lead to abnormalities in Ca2+ signaling through InsP3 channel in the cells, including cortical brain neurons. Finding a mechanism that relates mutant PS to exaggerated Ca2+ signaling may help us to attribute the pathological features of AD to this alteration in Ca2+ signaling. Therefore AD symptoms can be explained in a new way that may lead to therapeutic innovations for curing Alzheimer’s disease.

**Future Directions:**

• Researchers will probably do further investigations to find a cure for Familial Alzheimer’s disease by modification of the Ca signaling
• Now that the effect of