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Impaired mitochondrial function due to familial Alzheimer's disease-causing presenilins mutants via Ca^{2+} disruptions

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ABSTRACT

Mutants in presenilins (PS1 or PS2) is the major cause of familial Alzheimer's disease (FAD). FAD causing PS mutants affect intracellular Ca^{2+} homeostasis by enhancing the gating of inositol trisphosphate (IP_3) receptor (IP_3R) Ca^{2+} release channel on the endoplasmic reticulum, leading to exaggerated Ca^{2+} release into the cytoplasm. Using experimental IP_3R-mediated Ca^{2+} release data, in conjunction with a computational model of cell bioenergetics, we explore how the differences in mitochondrial Ca^{2+} uptake in control cells and cells expressing FAD-causing PS mutants affect key variables such as ATP, reactive oxygen species (ROS), NADH, and mitochondrial Ca^{2+}. We find that as a result of exaggerated cytosolic Ca^{2+} in FAD-causing mutant PS-expressing cells, the rate of oxygen consumption increases dramatically and overcomes the Ca^{2+} dependent enzymes that stimulate NADH production. This leads to decreased rates in proton pumping due to diminished membrane potential along with less ATP and enhanced ROS production. These results show that through Ca^{2+} signaling disruption, mutant PS leads to mitochondrial dysfunction and potentially to cell death.

1. Introduction

Alzheimer's disease (AD) is a form of dementia that is characterized by extensive synaptic and neuronal loss which leads to impaired memory and cognitive decline. Most AD cases are sporadic (SAD) and account for a large population of AD patients, but about 10% are inherited (FAD) and develop as early as the age of 30 as a result of mutations in amyloid precursor protein (APP) or presenilins (PS1, PS2) [7,23]. Both SAD and FAD share the features of accumulated extra- and intracellular β-amyloid plaques, intracellular neurofibrillary tangles composed mostly of hyperphosphorylated tau protein, and cell atrophy and death in multiple brain regions [21,29,44]. Both types of AD share similar phenotypes, suggesting a common pathogenic origin. However, mechanisms for how these proteins create such devastating effects in the neuron are still unclear. The observed Ca^{2+} signaling disruptions provides a potential mechanism for the common pathogenesis of both types of AD.

PS are transmembrane proteins that are synthesized on the endoplasmic reticulum (ER) [1]. PS forms a protein complex that is transported to the cell surface where it functions as a γ-secretase that cleaves type 1 transmembrane proteins, including APP [12]. The cleavage of APP releases Aβ peptides, which is a major component of amyloid plaques in the brains of patients with AD. These mutant PSs are thought to affect APP processing by increasing the production of amyloidogenic Aβ. Besides the disruption of APP processing, FAD-linked PS mutants dysregulate intracellular Ca^{2+} homeostasis [7,8,28]. PS mutants influence intracellular Ca^{2+} signaling by means of exaggerated Ca^{2+} release from the ER through the inositol 1,4,5-trisphosphate (IP_3) receptor (IP_3R), the main intracellular Ca^{2+} release channel in animal cells [7,8,19,22,30,45–47].

The Ca^{2+} hypothesis of brain aging and AD first proposed by Khatchaturian [26] suggests that over time, Ca^{2+} handling of neurons becomes compromised, leading to excessive free intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) and reactive oxygen species (ROS), mitochondrial dysfunction, and eventually apoptosis. Increasing data suggests that neuronal Ca^{2+} dysregulation plays a critical role in AD pathogenesis [4,48].

Mitochondria is responsible for the production of ATP, which is driven by the TCA cycle and oxidative phosphorylation. It is generally believed that mitochondria can function as a dynamic Ca^{2+}

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sequestration system [41]. Ca^{2+} plays a crucial role in facilitating the communication between mitochondria and other components of the cell [39]. Ca^{2+} has been shown to activate the mitochondrial matrix dehydrogenases in the TCA cycle [14,35] and influence the supply of NADH and $FADH_2$, thereby affecting the oxidative phosphorylation and the amount of ATP produced in the matrix. Thus, irregularities in mitochondrial Ca^{2+} homeostasis can cause severe complications to cellular physiological functions including bioenergetics.

Cheung et al. [7] observed that FAD-causing PS mutants lead to gain-of-function enhancement of IP_3R Ca^{2+} release channels. This gain-of-function enhancement at the single channel level leads to high-frequency, high-amplitude whole-cell Ca^{2+} oscillations in FAD-causing PS mutants-expressing cells as compared to control cells expressing wild-type (WT) PS1. Shilling et al. [44] showed that reversing such a gain-of-function enhancement of IP_3R by the genetic reduction of the IP_3R expression in PS1-M146V knock-in and $3\times$Tg mice models of FAD profoundly attenuated Aβ accumulation and tau hyperphosphorylation and rescued hippocampal long term potentiation memory deficits.

Here we investigate how these high-frequency and high-amplitude $[Ca^{2+}]_i$ oscillations impair mitochondrial function in cells expressing PS mutants. We achieve this by feeding experimental whole-cell $[Ca^{2+}]_i$ time traces directly into a computational model that takes into consideration the TCA cycle, oxidative phosphorylation, Ca^{2+} dynamics, and ROS production. We compare the mitochondrial function in a cell with PS mutant to that in a control cell. We show that the increased Ca^{2+} uptake in the mitochondria due to exaggerated Ca^{2+} release through IP_3R in FAD causing PS1 mutant-expressing cells leads to decreased ATP production, increased ROS in mitochondria ($[O_2^-]_m$) and $[H_2O_2]$, which over time can lead to cell death. The cells that we study are representatives of all the observations in [7] and the conclusions apply to all experiments in that study.

2. Materials and methods

2.1. Experimental methods

2.1.1. Intracellular calcium recordings

Whole-cell recordings obtained using fluorescence Ca^{2+} imaging of intracellular Ca^{2+} signals in human B lymphoblasts, elicited by exposure to a weak 50 ng/ml stimulation of human IgM, as reported in [7], were used in this study. Since IP_3 exerts no influence on the caffeine-induced, ryanodine-sensitive RyR Ca^{2+} channels, Ca^{2+}-release came through IP_3R. We compare the Ca^{2+} signals in human lymphoblasts expressing FAD-causing M146L mutant PS derived from FAD patients to signals in control lymphoblasts expressing WT PS derived from normal individuals to investigate the impact of mutation in PS on mitochondrial function. To demonstrate the robustness of our results we also study the affect of mutant PS1-A246 and PS2-NL141-induced $[Ca^{2+}]_i$ up-regulation on mitochondrial function (shown in Supporting Information). We refer the interested reader to [7] for information on how the cells were obtained, purified, and prepared.

2.2. Computational methods

Our model incorporates mitochondrial physiological processes such as the tricarboxylic acid (TCA) cycle, oxidative phosphorylation, Ca^{2+} signaling pathways, and ROS dynamics. We adopted the computational model from Cortassa et al. [9], which was modified from the work done by Magnus and Keizer [32]. The main components in our model are shown in Fig. 1. Rate equations for variables are given in Table S1 and their corresponding parameters and fluxes are described in the supporting information.

The model developed by Magnus and Keizer describes Ca^{2+} handling by mitochondria in the pancreatic β-cell. The Magnus and Keizer model includes 6 transport mechanisms in the inner mitochondrial membrane: proton pumping due to respiration, proton pumping uptake by the F_1F_0-ATPase, a proton leak, the adenine nucleotide exchanger, the Ca^{2+} uniporter, and the Na^+/Ca^{2+} exchanger. The original Magnus and Keizer model also includes dynamic changes in mitochondria membrane potential ($\triangle\Psi_m$), ADP ($[ADP]_m$), and Ca^{2+} concentration ($[Ca^{2+}]_m$) by means of 3 rate equations. The Magnus and Keizer model was expanded by Fall and Keizer [18] to include coupling with Ca^{2+} release through IP_3R based on the De Young-Keizer model that describes the properties of Ca^{2+} release from the ER through IP_3R channels [13]. In the Fall and Keizer model, Ca^{2+} released from the ER is sequestered into mitochondria through the Ca^{2+} uniporter [18]. The Fall and Keizer model was then expanded to include the mitochondria permeability transition pore (PTP) [15,39].

Missing in these studies is a mechanism through which Ca^{2+} regulates the TCA cycle and ROS production dynamics that is dependent upon oxygen consumption of the mitochondria. The work done by Cortassa et al. [9,10] includes TCA cycle dehydrogenases that drive NADH production, and ROS production due to a shunt of electrons from the electron transport chain. The Cortassa et al. model is a 16 variable model and shows that when Ca^{2+}-sensitive dehydrogenases are the main rate-controlling steps of respiratory flux, there are significant increases in oxygen consumption (V_{o_2}), proton efflux, NADH, and ATP synthesis. The Cortassa et al. model is able to reproduce experimental data concerning mitochondrial bioenergetics, Ca^{2+} dynamics, and respiration control [9]. Thus, we choose this model for our study although other expansions of the Magnus–Keizer model are also available in the literature [25,36,37]. Also, different versions of the model are used for mitochondrial bioenergetics in other cell types including Ehrlich ascites tumor cells and astrocytes [15,39] reflecting the robustness of the model and its ability to represent many cell types.

Our main goal in this study, is to combine experimental Ca^{2+} release from whole-cell cytosolic data from [7] and the Cortassa et al. model to compare the differences in mitochondrial bioenergetics in the absence and presence of PS mutant-induced exaggerated IP_3R-mediated cytosolic Ca^{2+} release. We remark that our conclusions about the impairment of mitochondrial function in the presence of PS-mutants qualitatively remain the same irrespective of the model selection. In the following we describe the key components of the model. The full details and equations of the model are given in supporting information.

2.2.1. TCA cycle

Isocitrate ([ISOC]), α-ketoglutarate ([αkg]), succinyl CoA ([SCoA]), succinate ([Suc]), fumarate ([FUM]), malate ([MAL]), and oxalacetate ([OAA]) are all chemical intermediates involved in the TCA cycle. The enzymes generating or processing these entities in the TCA cycle are citrate synthase (CS), aconitase (ACO), isocitrate dehydrogenase (IDH), alpha-ketoglutarate dehydrogenase (KGDH), succinyl CoA lyase (SL), succinate dehydrogenase (SDH), fumarate hydratase (FH), malate dehydrogenase (MDH), and aspartate amino transferase (AAT). The rate equations of the reactions catalyzed by these enzymes are shown in the supporting material. The TCA cycle provides a pathway for substrate oxidation. The cycle completes the oxidation of AcCoA to CO_2 and produces NADH and $FADH_2$. The TCA cycle enzymes isocitrate dehydrogenase (IDH) and alpha ketoglutarate dehydrogenase (KGDH) have explicit dependence on $[Ca^{2+}]_m$. The coefficients of the TCA cycle equations were determined in [9,32], and can be viewed in Table S2 and S3.