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Short communication

Familial Alzheimer's disease-linked presenilin-1 mutation M146V affects store-operated calcium entry: Does gain look like loss?

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ABSTRACT

Alzheimer's disease (AD) is a neurodegenerative disorder that leads to neuron death and synapse loss in the hippocampus and cortex, with consequent cognitive disability and dementia. Mutations in the presenilin-1 (PS1) gene lead to familial Alzheimer's disease (FAD). Here, we report that the expression of FAD-linked PS1 M146V mutant affects store-operated calcium channel activity (Isoc) in human neuroblastoma SK–N–SH cells. Electrophysiological measurements and calcium imaging experiments have revealed the emergent role of calcium sensor STIM2 in the inhibition of calcium release-activated calcium channel activity (Icrac) and enhancement of intracellular Ca^{2+} stores content due to PS1 M146V mutant expression. In general, the results of this study suggest that the pathological inhibition of one type of store-operated calcium channels caused by FAD PS1 mutant expression may be accounted for by preceding gain of spontaneous activity of store-operated calcium channels driven by STIM2.

1. Introduction

Alzheimer's disease (AD) is a neurodegenerative disorder that leads to neuron death and synapse loss in the hippocampus and cortex, with consequent cognitive disability and dementia. Mutations in presenilin-1 (PS1), presenilin-2 (PS2) and amyloid precursor protein (APP) lead to familial Alzheimer's disease (FAD). Most of FAD cases are connected with mutations in the PS1 gene. The hallmarks of AD include the presence of extracellular β-amyloid (Aβ) plaques and intracellular neurofibrillary tangles in the brain tissue, which are indicative of neuropathology [1]. It has been shown that disturbances in cell Ca^{2+} homeostasis are one of early pathological changes observed in AD patients [2,3]. Several studies have provided evidence for the impairment of store-operated calcium channels activity (Isoc) caused by FAD mutations in the PS1 gene [4–7]. Isoc is upregulated by calcium sensors STIM1 and STIM2. These sensors are signal transducers that trigger Ca^{2+} influx through SOC channels in the plasma membrane in response to depletion of Ca^{2+} stores in the endoplasmic reticulum (ER). STIM2, compared to STIM1, activates Isoc upon a smaller decrease in ER Ca^{2+}, since its EF-hand motif has a lower affinity for Ca^{2+} in the ER lumen [8]. Isoc contributes to basic neuronal functions, including long-term potentiation and neurotransmitter release [9,10]. PS1 mutation-driven disturbances of Isoc have been shown to affect APP processing, with the consequent increase in production of neurotoxic Aβ1-42 [11]. Therefore, an insight into the mechanism of Isoc impairment by PS1 mutations is important for developing new approaches to AD treatment.

As shown previously, the FAD-linked PS1 M146V mutation inhibits SOC entry [5,6,12], but there has been little agreement as to what mechanism underlies its effect. In particular, it has been proposed that this effect is connected with ER calcium leak and/or ER calcium load [12]. Here we present, for the first time, experimental evidence for the role of STIM2 ER calcium sensor in PS1 M146V mutant-driven effect on Isoc in neuronal cells, obtained by direct electrophysiological measurements.

2. Materials and methods

2.1. Cells and transient transfection

SK–N–SH human neuroblastoma cells (Cell Culture Collection, Institute of Cytology, St. Petersburg, Russia), cultured in DMEM with Penicillin/Streptomycin and 10% FBS, were transiently cotransfected with PS1 or PS1 M146V expression constructs in pcDNA3 vector and EGFP plasmid (molar ratio 5:1) using Lipofectamin-2000 (Invitrogen, United States). STIM2 shRNA and nontargeted shRNA plasmids in pRS vector were from OriGene Technologies (United States).

Abbreviations: FAD, familial Alzheimer's disease; PS1, presenilin-1; ER, endoplasmic reticulum; SOC, store-operated calcium; Tg, thapsigargin; Io, ionomycin.

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2.2. Electrophysiological measurements

Whole-cell recordings were made using an Axopatch 200B patch clamp amplifier and a Digidata 1332 A/D converter (Axon Instruments, United States). The composition of the pipette solution (mM) was 120 CsCl, 5 BAPTA-Na, 30 Cs-HEPES (pH 7.3), 4 Mg-ATP, 1.6 $CaCl_2$ (pCa 7.0); of the extracellular solution (mM), 140 NMDG-asp, 10 $BaCl_2$, 10 Cs-HEPES (pH 7,3), 0.01 Tetrodotoxin (Alomone Labs, Israel), 0.01 Nifedipine (Sigma, United States). Currents were sampled at 5 kHz and filtered digitally at 500 Hz. The pClamp9 software (Axon Instruments) was used for data acquisition and analysis. In all experiments, the holding potential was −40 mV. Once every 5 s, the membrane potential was stepped to −100 mV (for 30 ms) and a 200 ms voltage ramp to +100 mV was applied. Traces recorded before Isoc activation were used as a template for leak subtraction. The recorded currents were normalized relative to the cell capacitance, which averaged 19 ± 4 pF ($n = 30$).

2.3. Calcium imaging

Cells were loaded with 5 μM Fura-2AM in the presence of 0.025% Pluronic for 50 min at room temperature, in HBSS (mM): 130 NaCl, 5 KCl, 1.2 $MgCl_2$, 10 HEPES (pH 7.4), 1.8 $CaCl_2$. Loaded cells were illuminated by alternating 340- and 380-nm excitation light at 2 Hz. Emission fluorescence intensity was measured at 510 nm with the InCyt Basic I/P dual wavelength fluorescence imaging system (Intracellular Imaging Inc., United States). The change in cytosolic Ca^{2+} concentration was expressed as the ratio of emission fluorescence intensities at 340 and 380 nm excitation wavelengths (340/380-nm ratio). The composition of Ca-free solution (mM) was 130 NaCl, 5 KCl, 1 $MgCl_2$, 20 HEPES/KOH (pH 7.3), 0.2 EGTA.

2.4. Western blotting

Total cells lysates separated by 8% SDS-PAGE were transferred onto Immobilon P membrane (Millipore Inc., United States), treated with primary anti-STIM2 antibody (Alamone Labs) and secondary anti-rabbit or anti-mouse antibody (Sigma), and developed with SuperSignal Chemiluminescent Substrate (Pierce, United States). Anti-α-tubulin monoclonal antibody (Sigma) was used to test for equal protein loading. Treatment with antibodies was performed according to the manufacturer's protocols.

3. Results and discussion

3.1. PS1 M146V mutant decreases thapsigargin-induced Isoc in human neuroblastoma SK–N–SH cells

The effect of PS1 M146V mutation on Isoc was analyzed in a series of electrophysiological experiments with SK–N–SH human neuroblastoma cells transiently cotransfected with wild-type (WT) human PS1 or mutant PS1 M146V and EGFP expression constructs. The transfected cells were identified by GFP fluorescence. In whole cell recordings, passive Ca^{2+} store depletion after addition of 1 mM thapsigargin (Tg) induced inward cation currents with a linear *I*/*V* (current/voltage) relationship and a reversal potential (Erev) around +30 mV (Fig. 1A, B). The peak amplitudes of Isoc inward currents in nontransfected cells and in cells transfected with PS1 WT were found to be almost the same: −1.55 ± 0.242 pA/pF and −1.85 ± 0.370 pA/pF, respectively, at a holding potential of −80 mV; compared to these values, the amplitudes of Tg-induced Isoc currents in cells with PS1 M146V expression were significantly lower, reaching a maximum of only −0.61 ± 0.201 pA/pF at −80 mV (Fig. 1A, B). The decreased Isoc activity observed in electrophysiological experiments with neuronal cells transfected with the PS1 M146V expression plasmid is consistent with previous data on the decreased SOC entry obtained in Ca^{2+} imaging experiments with hippocampal neurons of triple-transgenic AD mice (KI–PS1 M146V, Thy1–APPKM670/671NL, Thy1–tauP301L), transfected SY5Y cells, and PS1 M146V-KI fibroblasts [5,6,12].

3.2. STIM2 knockdown rescues Isoc amplitude and attenuates intracellular calcium load in cells expressing PS1 M146V

As hypothesized previously, ER calcium overload is the factor responsible for Isoc reduction [5,12]. Both STIM1 and STIM2 sensors trigger Ca^{2+} influx through SOC channels in response to depletion of ER Ca^{2+} stores, but STIM2, compared to STIM1, activates this influx upon a smaller decrease in ER Ca^{2+}, since its EF-hand motif has a lower affinity for Ca^{2+} in the ER lumen [8]. Therefore, we supposed that STIM2 could still activate SOC currents in cells with PS1 M146V mutant expression, with all detectable currents being operated by STIM2. To test this hypothesis, we used the shRNA approach to knockdown STIM2 expression, verifying the knockdown by Western blotting (Fig. 2C). STIM2 knockdown in control cells expressing PS1 WT did not lead to any changes in Tg-induced currents (Fig. 2A, B).

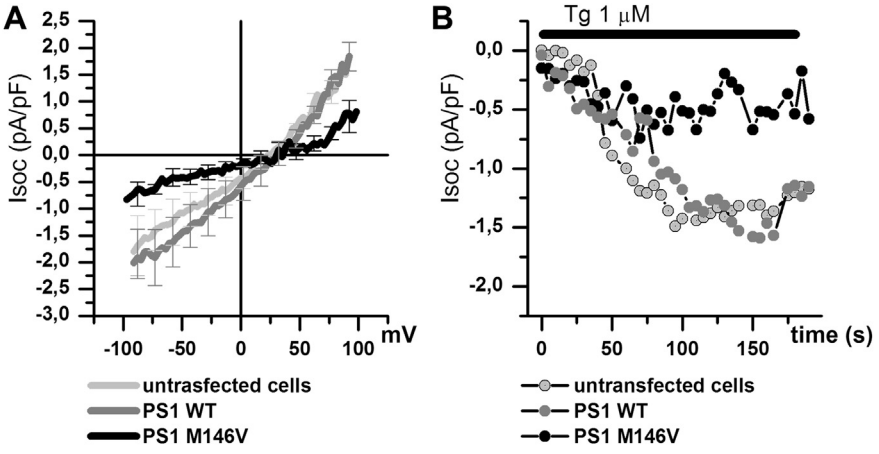

Fig. 1. Reduction of thapsigargin-induced Isoc currents in whole cell recordings from SK–N–SH cells transfected with PS1 M146V mutant. (A) Current/voltage (*I*/*V*) relationships for Isoc evoked by 1 μM thapsigargin (Tg) in SK–N–SH cells transfected with PS1 WT or PS1 M146V and in untransfected cells (mean ± SEM; $n = 5–8$, $p < 0.05$). Measurements were made when the currents reached a maximum. (B) Amplitudes of Isoc currents in SK–N–SH cells transfected with PS1 WT or PS1 M146V and in untransfected cells as a function of time after application of 1 μM Tg. Measurements were made at each ramp at −80 mV potentials. The results of representative experiments are shown.

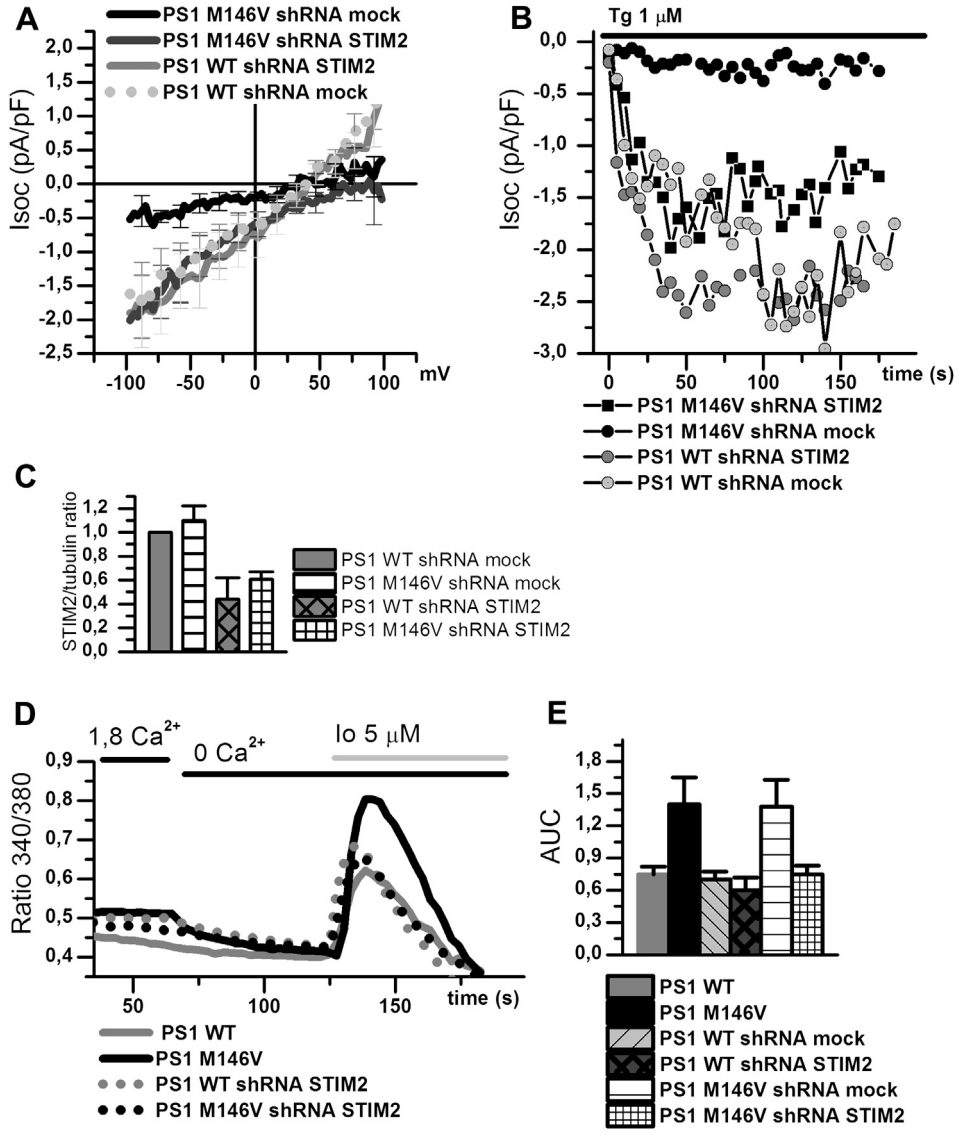

Fig. 2. STIM2 knockdown rescues the amplitude of Isoc currents and attenuates ER calcium load in SK–N–SH cells transfected with PS1 M146V. (A) Current/voltage (*I*/*V*) relationships for Isoc evoked by 1 μM thapsigargin (Tg) in SK–N–SH cells transfected with PS1 WT or PS1 M146V and anti-STIM2 shRNA (shRNA STIM2) or untargeted shRNA (shRNA mock) (mean ± SEM; $n = 4–9$, $p < 0.05$). Measurements were made when the currents reached a maximum. (B) Amplitudes of Isoc currents in SK–N–SH cells transfected with PS1 WT or PS1 M146V and anti-STIM2 shRNA or untargeted (mock) shRNA as a function of time after application of 1 μM Tg (indicated by line). Measurements were made at each ramp at −80 mV potentials. The results of representative experiments are shown. (C) Relative levels of STIM2 in SK–N–SH cells transfected with PS1 WT or PS1 M146V and anti-STIM2 shRNA or untargeted (mock) shRNA as estimated by immunoblotting with polyclonal anti-STIM2 and monoclonal anti-α-tubulin antibodies. Tubulin levels were used as loading and specificity controls. The amount of STIM2 was quantified by densitometry, normalized relative to the amount of α-tubulin detected in each lane, and plotted as a proportion of STIM2 amount in the variant PS1 WT shRNA mock ($n = 3$, $p < 0.05$) (D) Fura-2 340/380-nm ratio traces characterizing ionomycin-sensitive Ca^{2+} pools in SK–N–SH cells transfected with PS1 WT or PS1 M146V alone or with anti-STIM2 shRNA or untargeted (mock) shRNA. After incubation in HBSS, the cells were transferred to Ca^{2+}-free medium for 60 s and challenged with 5 μM ionomycin (Io). Traces are based on mean values ($n = 141–162$ cells). (E) Average sizes of ionomycin-sensitive Ca^{2+} pools in transfected cells estimated as area under curve (AUC) of Io-induced Fura-2 signal. The AUC was integrated for each cell. The data from different experiments were averaged and presented as mean ± SEM ($n = 141–162$ cells, $p < 0.05$).

Unexpectedly, the amplitude of Tg-induced Isoc in cells with PS1 M146V mutant expression proved to increase after STIM2 knockdown, remaining unchanged after transfection with nontargeted shRNA, in addition to the PS1 mutant-expressing vector (Fig. 2A, B). Moreover, STIM2 knockdown in cells expressing PS1 M146V caused significant changes in the shape of Isoc *I*/*V* relationship: it was characterized by a strong inward rectification and an Erev of about +60 mV, which are hallmarks of Icrac store-operated calcium channels driven by STIM1 (Fig. 2A). Changes in *I*/*V* curve clearly shows that currents of nonselective channels were substituted with Icrac in Tg-induced SOC current. From this it follows that STIM2 may drive its function mainly through the nonselective SOC channels (such as formed by TRPC subunits) in cells with PS1 mutant expression, in contrast to control cells with PS1 WT.

It has recently been proposed that STIM2 regulates ER calcium level in neurons by stimulating the spontaneous activity of store-operated calcium channels, independently of STIM1 [13]. We used calcium imaging experiments to estimate the total Ca^{2+} store content by inducing Ca^{2+} efflux from stores to the cytoplasm with ionomycin. The expression of PS1 M146V mutant proved to result in an increase in the ionomycin-sensitive Ca^{2+} pool, compared to cells expressing PS1 WT, and the excessive rise in cytoplasmic Ca^{2+} concentration was prevented by STIM2 knockdown, with non-targeted shRNA having no such effect (Fig. 2D, E). The ionomycin-

sensitive Ca^{2+} pool in cells expressing PS WT remained unchanged either after STIM2 knockdown or in the experiment with non-targeted shRNA (Fig. 2D, E).

Hence, it appears that PS1 M146V expression is associated with spontaneous store-operated calcium channels activity driven by STIM2, which provides for the maintenance of high ER calcium level. As STIM1 sensor has lower sensitivity to changes in ER calcium concentration and acts in an all-or-nothing way [8], the ER store depletion with Tg is insufficient for STIM1 activation in cells expressing the mutant PS1. In this case, the STIM2-driven store-operated calcium channels gain-of-function plays the role of an "inhibitor" of STIM1-driven store-operated calcium entry (for example Icrac). An inhibition role of enhanced STIM2 signaling has been described in Refs. [8,14,15].

4. Conclusions

This study provides strong evidence, based on direct electrophysiological recordings, that the expression of FAD-linked mutant PS1 does affect the activity of store-operated calcium channels. The most important finding is that the effect of PS1 M146V is driven by STIM2 sensor. The detectable inhibition of Tg-induced store-operated calcium currents may result from preceding abnormal spontaneous activity of nonselective store-operated calcium channels activated by STIM2. Further study is needed to find out whether store-operated channel subunits, such as Orai or TRPC, are involved in the observed effect. The results described above may provide a basis for future research, in particular, may redirect the vector of drug screening for FAD treatment.

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