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The E280A presenilin mutation reduces voltage-gated sodium channel levels in neuronal cells

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Abstract

Background—Familial Alzheimer's Disease (FAD) mutations in presenilin (PS) modulate PS/ γ -secretase activity and therefore contribute to AD pathogenesis. Previously, we found that PS/ γ -secretase cleaves voltage-gated sodium channel β 2 subunits ($\text{Na}_v\beta$ 2), releases the intracellular domain of $\text{Na}_v\beta$ 2 (β 2-ICD), and thereby increases intracellular sodium channel α -subunit Na_v 1.1 levels. Here, we tested whether FAD-linked PS1 mutations modulate $\text{Na}_v\beta$ 2 cleavages and Na_v 1.1 levels.

Objective—To analyze the effects of PS1-linked FAD mutations on $\text{Na}_v\beta$ 2 processing and Na_v 1.1 levels in neuronal cells.

Methods—We first generated B104 rat neuroblastoma cells stably expressing $\text{Na}_v\beta$ 2 and wild-type PS1, PS1 with one of three FAD mutations (E280A, M146L, or Δ -E9), or PS1 with a non-FAD mutation (D333G). $\text{Na}_v\beta$ 2 processing and Na_v 1.1 protein and mRNA levels were then analyzed by Western blot and real-time RT-PCR, respectively.

Results—The FAD-linked E280A mutation significantly decreased PS/ γ -secretase-mediated processing of $\text{Na}_v\beta$ 2 as compared to wild-type PS1 controls, both in cells and a cell-free system. Na_v 1.1 mRNA and protein levels, as well as the surface levels of Na_v channel α -subunits, were also significantly reduced in PS1 (E280A) cells.

Conclusion—Our data indicate that the FAD-linked PS1 E280A mutation decreases Na_v channel levels by partially inhibiting PS/ γ -secretase-mediated cleavage of $\text{Na}_v\beta$ 2 in neuronal cells.

Keywords

Alzheimer's disease; presenilin; voltage-gated sodium channel

Introduction

Mutations in the presenilin 1 gene (PSEN1) are the most common genetic cause of Familial Alzheimer Disease (FAD)[1]. PS1 mutations are likely to exert their pathophysiological effect by altering PS1/ γ -secretase-mediated cleavage of the amyloid- β precursor protein (APP), resulting in increased $\text{A}\beta_{42/40}$ ratios [2, 3]. PSEN1 FAD patients are affected by early onset cognitive decline typical of AD, but additional symptoms are also present in patients

with select FAD mutations [4]. These include myoclonus and/or seizures, apraxia, dystonia, and behavioral and psychiatric symptoms [4-6]. However, molecular mechanisms underlying these atypical symptoms are not fully understood.

Previously, we and others reported that the $\beta 2$ -subunit of the voltage-gated sodium channel ($\text{Na}_v\beta 2$) is a substrate of BACE1 and PS/ γ -secretase [7-10]. We found that PS/ γ -secretase-mediated cleavages of $\text{Na}_v\beta 2$ C-terminal fragments ($\beta 2$ -CTFs) released the intracellular domains of $\text{Na}_v\beta 2$ ($\beta 2$ -ICDs), which was localized to the nucleus and increased mRNA and protein levels of a Na_v channel α -subunit, $\text{Na}_v1.1$ [7-9, 11]. These studies clearly demonstrate that PS/ γ -secretase-mediated processing of $\text{Na}_v\beta 2$ plays a major role in regulating neuronal sodium channel $\text{Na}_v1.1$. Since Na_v1 channels play an important role in neural activity, here we tested if specific PS1 FAD mutations contribute to abnormal $\text{Na}_v1.1$ levels by altering $\text{Na}_v\beta 2$ processing.

Methods

Constructs, transfection, and cell lines

The construct encoding full-length human $\text{Na}_v\beta 2$ containing a C-terminal V5/His tag was described previously [8]. Constructs containing human wild-type PS1 (wtPS1) and PS1(Δ E9) and PS1(D333G) were a kind gift from Dr. John Hardy (NIH). For PS1(E280A) and PS1(M146L) constructs, mutations were introduced into wtPS1 by site-directed mutagenesis (Promega). Effectene (Qiagen) was used for transfection and individual clones were maintained in selection medium containing Zeocin (Invitrogen) and G418 (CellGro).

Western Blot analysis, antibodies, and inhibitors

Cell extraction and Western blot analyses were performed as previously described [7]. Primary antibodies were used at the following dilutions: anti-V5 (1:5000, Invitrogen), anti- $\text{Na}_v1.1$ (1:250, Millipore), anti-pan $\text{Na}_v1 \alpha$ (1:250, Millipore), anti-PS1 (1:250, Millipore), anti-GAPDH (1:250, Millipore), anti-NCadherin (1:1000, BD Transduction Laboratory), and anti-transferrin receptor (1:1000, Sigma). The γ -secretase inhibitors DAPT and L-685,458 were obtained from Calbiochem. Blots were quantified using the Versa Doc imaging system and Quantity One software (Biorad).

Cell-free γ -secretase cleavage assay

In vitro γ -secretase assay was performed as previously described [8]. Purified membrane fractions were resuspended in a reaction buffer and incubated at 0 or 37 °C for two hours in the presence or absence of L-685,458.

RNA Extraction, cDNA synthesis, and quantitative real-time RT-PCR

Relative $\text{Na}_v1.1$ mRNA levels were measured by quantitative real-time RT-PCR as previously described [7]. All experiments were normalized to GAPDH controls.

Cell Surface Biotinylation

Cell surface biotinylation and detection of surface $\text{Na}_v1 \alpha$ -subunits were performed as previously described [7, 12].

Results and Discussion

To study the effects of PS1 mutations on Na_v1 channels, we stably cotransfected B104 rat neuroblastoma cells with $\text{Na}_v\beta 2$ and wtPS1 or PS1(E280A or M146L), both FAD mutations associated with clinical seizures or PS1(Δ E9), a FAD mutation with no reported seizures, or

PS2(D333G), a non FAD-linked mutation [4, 5]. B104 cells express endogenous Na_v1 channel components and have previously been used for studying Na_v1.1 channel regulation by BACE1 and PS/γ-secretase [7]. In these cells, we first analyzed levels of β2-CTF, a direct substrate for PS/γ-secretase (**supplemental Fig. 1a**). Interestingly, we found that a seizure-related FAD-linked PS1 mutation, E280A increased β2-CTF levels by 3.7 fold as compared to the control cells (**Fig. 1a** and **supplemental Fig. 1b**). We also observed a moderate increase of β2-CTF levels (1.9 fold) in PS1(M146L) cells but this did not reach statistical significance (**Fig. 1a** and **supplemental Fig. 1b**). Ectodomain shedding by ADAM10 or BACE1 was not increased, since we were unable to detect elevated β2-CTF levels in the same cells pretreated with the PS/γ-secretase inhibitor DAPT (**Fig. 1a**). This indicates that the E280A mutation prevents PS/γ-secretase cleavage of Na_vβ2.

To confirm this, we quantitated levels of the PS/γ-secretase cleavage product β2-ICD (**Fig. 1b** and **c**). Since β2-ICDs are quickly degraded within the cytoplasm, a cell-free γ-secretase cleavage assay system (**Fig. 1b**) or overexpression of recombinant myc-tagged β2-CTF substrate (**Fig. 1c**) are required to detect β2-ICDs. Both assays showed a dramatic decrease in β2-ICDs with PS1(E280A) expression (**Fig. 1b, c**). Steady state levels of myc-β2-CTF were largely elevated in PS1 (E280A) cells as compared to wtPS1 controls (**supplemental Fig. 2b** and **c**). Together, these data demonstrate that the PS1 E280A mutation decreases PS/γ-secretase-mediated Na_vβ2 cleavages in neuronal cells.

Next, we tested whether the PS1 E280A mutation reduces Na_v1.1 levels by decreasing β2-ICD levels. By using quantitative real-time RT-PCR, we analyzed Na_v1.1 mRNA levels of PS1(E280A) and wtPS1 cell clones (n= 4 for PS1(E280A) and 3 for wtPS1). We found that Na_v1.1 mRNA levels are dramatically decreased in PS1(E280A) cells as compared to wtPS1 cells, which is consistent with decreased β2-ICD levels in these cells (**Fig. 2a**). As expected, we also found that Na_v1.1 protein levels were significantly decreased in PS1(E280A) cells (**Fig. 2b**). These data strongly indicate that the PS1 E280A mutation decreased Na_v1.1 levels by reducing β2-ICD generation in these cells.

To test whether the decrease in Na_v1.1 levels led to a reduction in functional Na_v1 channels, we analyzed cell surface levels of Na_v1 α-subunits (**Fig. 2c** and **d**). We have previously shown that decreases in surface Na_v1 α-subunit levels were directly responsible for decreased sodium currents in the same cells [7]. By using a cell surface biotinylation assay, we found that surface Na_v1 α-subunit levels were dramatically decreased in PS1(E280A) cells as compared to wtPS1 controls (**Fig. 2c** and **d**). Pharmacological inhibition of PS/γ-secretase activity confirmed our data with PS1(E280A) overexpression, as DAPT robustly reduced surface Na_v1 α-subunit levels in B104 cells expressing Na_vβ2 without PS1(E280A) expression (**Supplemental Fig. 3**). Together, our results indicate that PS1 E280A mutation decreased PS/γ-secretase-mediated cleavage of Na_vβ2, β2-ICD generation, Na_v1.1 α-subunit precursor levels, and therefore reduced the surface Na_v1 channels and possibly sodium currents and membrane excitability (**Fig. 3**).

Recently, we reported that BACE1 ablation decreased Na_v1.1 mRNA and proteins levels in mouse brains due to reduced endogenous β2-ICD levels [13]. Consistent with our findings, Hu *et al.* also showed that Na_v1.1 and Na_v1.2/6 levels were significantly decreased in BACE1-null mice, although the changes in Na_v1.2/6 were not observed in other reports [14, 15]. A recent study in the PS/APP AD mouse model also showed that the elevated BACE1 levels in these mice led to the accumulation of endogenous β2-ICD in nuclei of cortical neurons, increased, Na_v1.1 levels, and possibly contributed to aberrant neuronal activity and cognitive deficits [16]. Together, these data strongly support a physiological role of β2-ICD in regulating Na_v1.1 levels in brains.

AD patients with PS1 E280A mutation display frequent epileptic seizures at the early ages and a study showed that the early onset seizures in PS1 E280A patients strongly associated with CA1 hippocampal neuronal loss [5, 17, 18]. Recent studies in AD mouse models also provide strong evidence that altered Na_v1.1 channel levels are responsible for seizures, abnormal neural activities and even a cognitive decline in these mice [16, 19]. Therefore, our findings suggest a novel molecular pathway that can contribute to AD pathology in patients with PS1 E280A mutation. Further studies will be required to fully elucidate whether PS1(E280A) and possibly other epilepsy-associated PS FAD mutations contribute to AD pathology by modulating Na_v1.1 channels *in vivo*.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Fig 1.

PS1(E280A) partially inhibits γ -secretase-mediated $\text{Na}_v\beta 2$ processing. **a** Western blot analysis of $\text{Na}_v\beta 2$ full-length and CTF levels in stable cell lines co-expressing $\text{Na}_v\beta 2$ and wild type PS1 (wtPS1) or PS1 containing E280A, M146L, Δ E9 or D333G mutations. Cells were pretreated with DMSO or 500 nM DAPT for 24 hrs. **b, c** The PS1(E280A) mutation also decreased $\beta 2$ -ICD generation in a cell-free γ -secretase cleavage assay (**b**) and in membrane extracts from PS1(E280A) cells coexpressing a recombinant myc- $\beta 2$ -CTF (**c**).

Fig 2.

PS1(E280A) decreased $\text{Na}_v1.1$ mRNA and protein levels. **a** Relative $\text{Na}_v1.1$ mRNA levels were measured by real-time RT-PCR in all clones stably expressing $\text{Na}_v\beta 2$ and PS1 with or without E280A mutation (*, $P < 0.05$; $n = 3$ for wtPS1 and 4 for PS1(E280A); Student's *t*-test). **b** Western blot analyses of $\text{Na}_v1.1$ levels in select stable cell lines expressing similar levels of $\text{Na}_v\beta 2$ and wtPS1 or PS1(E280A). **c** Surface levels of sodium channel α -subunits were significantly decreased in PS1(E280A) cells as compared to wtPS1 control cells. **d** Quantitation of **c** (*, $P < 0.05$; $n = 3$; Student's *t*-test).

Fig 3. Diagram illustrating how the PS1 E280A mutation regulates voltage-gated sodium channel levels.