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γ -Secretase Activity Modulates Store-Operated Ca^{2+} Entry into Rat Sensory Neurons

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Abstract

Presenilin-1 is required for γ -secretase activity, which participates in Notch receptor processing, the pathogenesis of Alzheimer's disease and the modulation of Ca^{2+} signaling. We tested the hypothesis that γ -secretase proteolytic activity modulates store-operated Ca^{2+} entry (SOCE) in rat dorsal root ganglion neurons. Depletion of intracellular Ca^{2+} stores by blocking the endoplasmic reticulum Ca^{2+} pump with cyclopiazonic acid (CPA) evoked a transient increase in $[\text{Ca}^{2+}]_i$ but no sustained Ca^{2+} influx. However, in cells expressing a dominant negative presenilin-1 mutant (PS1-D257A), γ -secretase activity was inhibited and treatment with CPA evoked sustained Ca^{2+} influx. Similarly, pharmacologic inhibition of γ -secretase with DAPT for 48 hrs enhanced SOCE. SKF96365, an inhibitor of store-operated channels, blocked SOCE in cells expressing PS1-D257A. Thus, γ -secretase proteolytic activity regulates a SOCE pathway in sensory neurons.

Keywords

γ -secretase; dorsal root ganglion neuron; capacitative Ca^{2+} entry; store-operated Ca^{2+} entry

The proteolytic enzyme γ -secretase participates in Notch receptor processing [10], the pathogenesis of Alzheimer's disease [17], and has recently been implicated in modulating Ca^{2+} signaling processes [26]. Dominant negative mutations in presenilin, which is required for γ -secretase activity, prevent the proteolytic processing of the Notch receptor resulting in developmental defects [14]. Mutations in the presenilins (PS1 and PS2) are the most common cause of early-onset familial Alzheimer's disease (FAD); they produce a gain-of-function that increases the level of amyloid- β 42 [29]. Presenilins are integral membrane proteins found predominantly in the endoplasmic reticulum (ER) and are also present in mitochondria, golgi, endosomes, and the intracellular side of the plasma membrane [23]. Cells that express presenilin mutants also exhibit changes in store-operated Ca^{2+} entry (SOCE) [19,11,34].

SOCE results from opening plasma membrane Ca^{2+} channels following depletion of intracellular Ca^{2+} stores. Inhibition of ER Ca^{2+} pumps or activation of ER Ca^{2+} release channels produces a drop in ER Ca^{2+} sensed by stromal interacting molecule (STIM1), which redistributes in the ER to activate plasma membrane Ca^{2+} channels such as Orai1 and TRPC [12,24]. SOCE was first described as a major route of Ca^{2+} influx in non-excitable cells and

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is now known to occur in excitable cells including neurons [3,28,31]. SOCE prolongs the elevation in $[Ca^{2+}]_i$ that follows store mobilization and, depending on cell type, enhances secretion [9], activates transcription [25], alters synaptic transmission [2] and assists in refilling depleted Ca^{2+} stores [8].

Fibroblasts from FAD PS1 knock-in mice and neuronal cells expressing FAD PS1 mutants exhibit a marked impairment in SOCE and increased Ca^{2+} levels within the ER [19,11,34]. PS1 modulation of SOCE is independent of the expression of amyloid precursor protein [11] but, PS1-dependent effects on phosphoinositide-mediated Ca^{2+} signals are mediated through changes in γ -secretase activity [27]. Cortical neurons from PS1 knockout mice display a marked potentiation in SOCE [34]. Expression of a dominant negative PS1 mutant (PS1-D257A) produces a variety of effects including loss of γ -secretase activity, PS1 endoproteolysis, and impaired notch signaling [14,33]. In neuronal cell lines expressing PS1-D257A or treated with a γ -secretase inhibitor, SOCE was potentiated and phosphoinositide-mediated elevations in $[Ca^{2+}]_i$ were inhibited [34,27].

Presenilin function has not been studied in sensory neurons and little is known about the regulation of SOCE in peripheral neurons. Because the mechanisms for regulating $[Ca^{2+}]_i$ in peripheral neurons differ from those in central neurons, especially concerning the role of intracellular Ca^{2+} stores, we examined whether presenilin-sensitive SOCE is expressed in sensory neurons. We found that genetic and pharmacologic inhibition of γ -secretase significantly enhanced SOCE in dorsal root ganglion (DRG) neurons.

Rat DRG neurons were grown in primary culture as described previously [31] under a protocol approved by the University of Minnesota Institutional Animal Care and Use Committee. $[Ca^{2+}]_i$ was recorded from single DRG neurons using indo-1 based photometry as previously described [32].

We used biolistic particle-mediated gene transfer [30] to express wild-type human PS1 (PS1-WT) and dominant negative PS1-D257A [33] in DRG neurons. Neurons were co-transfected with an enhanced green fluorescent protein (EGFP) expression vector in order to identify cells expressing the PS1 constructs. Cells transfected with EGFP expression vector alone served as controls (Sham). PS1-D257A inhibits γ -secretase activity [33] and as such decreases the production of the C-terminal fragment of beta-amyloid (AICD) and the C-terminal Notch fragment (NICD) [14]. Both AICD and NICD bind the transcriptional repressor CBF1 resulting in the activation of CBF1-repressed genes. Thus, to verify whether expression of D257A-PS1 inhibited γ -secretase activity in DRG neurons, we used a CBF1 luciferase reporter assay (4xwtCBF1Luc) [13]. 4xwtCBF1Luc was co-transfected with a plasmid containing *Renilla* luciferase under control of the CMV promoter (pRL-CMV), and the expression of firefly luciferase was normalized to constitutively expressed *Renilla* luciferase activity to correct for differences in transfection efficiency. γ -secretase cleavage products remove CBF1 repression, therefore a decrease in γ -secretase activity results in a reduction in normalized luciferase activity. As shown in Fig. 1A, cultures transfected with the PS1-D257A expression vector (n=3 coverslips) had $10 \pm 8\%$ of the γ -secretase activity of sham transfected cultures (n=3 coverslips). Luciferase expression in PS1-WT (n=3 coverslips) and sham-transfected neurons were not significantly different. These results demonstrate that PS1-D257A expression in rat DRG neurons inhibits endogenous γ -secretase activity.

The effect of PS1-D257A expression on SOCE in DRG neurons has not been studied. Figure 1B shows a representative SOCE response from a PS1-D257A-expressing cell evoked by continuous treatment with the sarcoplasmic-endoplasmic reticulum Ca^{2+} ATPase (SERCA) inhibitor, cyclopiazonic acid (CPA, 5 μ M) in the presence of extracellular Ca^{2+} . CPA blocks Ca^{2+} uptake into the ER resulting in release of Ca^{2+} into the cytoplasm producing a transient

rise in $[Ca^{2+}]_i$. The $[Ca^{2+}]_i$ then recovers to a sustained plateau maintained by SOCE. Analysis of basal, peak and plateau $[Ca^{2+}]_i$ from sham, PS1-WT and PS1-D257A transfected cells revealed that neither the resting $[Ca^{2+}]_i$ nor the peak amplitude of the CPA-induced response was significantly different among the groups (Fig. 1C). However, the plateau of the SOCE response, measured 2000 s after application of CPA, was elevated in PS1-D257A relative to either Sham or PS1-WT expressing neurons. The average plateau $[Ca^{2+}]_i$ in PS1-D257A was 55 % higher than sham and 98 % greater than PS1-WT expressing neurons (Fig. 1C). The representative traces in Figure 1D show that the $[Ca^{2+}]_i$ increase in PS1-D257A expressing cells was more sustained. The time to recover to 75% of the peak took 236 ± 34 s in neurons expressing PS1-D257A compared to 150 ± 27 s in PS1-WT and 180 ± 23 s in sham expressing neurons (Fig. 1E). Because these experiments were run in the continuous presence of CPA, the prolonged $[Ca^{2+}]_i$ increase observed in the PS1-D257A expressing neurons was independent of intracellular store-mediated Ca^{2+} uptake and release, suggesting the differences are likely due to prolonged activation of a Ca^{2+} influx pathway.

SOCE can also be studied by depleting the ER of Ca^{2+} and then initiating Ca^{2+} influx by return of Ca^{2+} to the medium. The effects of PS1-D257A expression on SOCE in DRG neurons were determined using a protocol slightly modified from one previously described [31]. Voltage-operated Ca^{2+} influx was evoked by depolarization with 30 mM K^+ (Fig. 2A). PS1-D257A expression did not affect resting $[Ca^{2+}]_i$ or the depolarization-induced $[Ca^{2+}]_i$ increase (Fig. 2C). After allowing the cell to recover for 15 minutes, 5 μ M CPA was applied for 5 minutes in Ca^{2+} -free medium (100 μ M EGTA) to deplete intracellular Ca^{2+} stores. CPA was then removed in the absence of extracellular Ca^{2+} . The return of extracellular Ca^{2+} (1.26 mM) produced an increase in $[Ca^{2+}]_i$ mediated by SOCE. Expression of PS1-D257A did not affect the peak amplitude of the SOCE response (Fig. 2C). In Figure 2B the effects of PS1-D257A (n=6) expression on the shape of the SOCE response are compared to sham (n=6) transfected neurons. The duration of the SOCE-mediated $[Ca^{2+}]_i$ increase was significantly prolonged in neurons expressing PS1-D257A. 500 s after returning Ca^{2+} to the extracellular medium, $[Ca^{2+}]_i$ was 63 ± 10 nM higher in the PS1-D257A expressing neurons compared to Sham transfected neurons (Fig. 2C). Furthermore, neurons expressing PS1-D257A took 207 ± 23 % longer to recover to 75 % of peak amplitude compared to sham cells (Fig. 2D). These data suggest that PS1-D257A expression prolongs SOCE in DRG neurons without affecting voltage-operated Ca^{2+} influx or Ca^{2+} clearance.

To address the question of whether PS1-D257A prolonged SOCE by virtue of its ability to inhibit proteolysis, we used an alternative pharmacological approach to inhibit γ -secretase activity. The cell-permeable, dipeptide compound DAPT blocks γ -secretase-mediated proteolysis [7]. We again used the CBF1 luciferase reporter assay to measure γ -secretase activity. As shown in Fig. 3A, 48 hr treatment of DRG neurons with 1 μ M DAPT (n=7) did not significantly affect luciferase expression, whereas treatment with 100 μ M DAPT (n=6) reduced transactivation of the CBF1-luciferase reporter by 45%. Treatment with 1 μ M DAPT did not significantly affect SOCE (n=7). However, 48 hr treatment with 100 μ M DAPT significantly enhanced SOCE compared to vehicle-treated control cells (n=9; Fig. 3B,C). $[Ca^{2+}]_i$ was 104 % higher in DAPT (100 μ M) treated neurons 2000 s after evoking SOCE. Basal $[Ca^{2+}]_i$ and the peak of the CPA-evoked response were not affected by DAPT. Treatment with DAPT 5 min prior to and during the recording did not affect the CPA-evoked response at 2000 s.

SKF96365 inhibits SOCE [22]. Here, we determined the contribution of SKF96365-sensitive ion channels to the sustained elevation in $[Ca^{2+}]_i$ induced by CPA. SKF96365 completely blocked SOCE in DRG neurons expressing PS1-D257A, as indicated by the rapid return of the CPA response to basal $[Ca^{2+}]_i$ (Fig. 4A). Thus, 2000s after CPA application the $[Ca^{2+}]_i$ in SKF96365-treated PS1-D257A-expressing cells (n=5) was significantly reduced relative to

PS1-D257A-expressing cells in the absence of drug (n=5)(Fig. 4C). SKF96365 evoked complex effects in DAPT treated cells (Fig. 4B). After an initial rapid recovery toward basal levels, $[Ca^{2+}]_i$ steadily rose for the remainder of the recording (n=13). The increase in $[Ca^{2+}]_i$ evoked by SKF96365 did not require store-depletion as it was also observed in DAPT-treated cells in the absence of CPA (n=11).

We have shown that inhibition of presenilin dependent proteolytic activity enhances SOCE in rat sensory neurons. These results are in good agreement with previous studies examining the role of presenilins in Ca^{2+} signaling. There is general consensus that FAD mutations in presenilin alter γ -secretase activity resulting in decreased SOCE and that inhibition of γ -secretase activity by dominant negative presenilins, such as the D257A mutant studied here, result in increased SOCE [6,19,34]. We did not observe a change in store capacity, as measured by the amplitude of the CPA-induced response, which differs from other reports [5]. Store refilling may depend on cell-specific factors such as other Ca^{2+} sources and SERCA activity.

Expression of PS1-D257A inhibited γ -secretase as indicated by reduced gene expression driven by the intracellular signaling domain. The marked changes in ion channel function following inhibition of γ -secretase raise some concerns about potential side effects should pharmacologic inhibitors be used to reduce β -amyloid production in Alzheimer's disease. Whether changes in γ -secretase activity are directly responsible for altered signaling in cells expressing mutant presenilins is controversial [11]. Changes in Ca^{2+} homeostasis resulting from dominant negative PS1 have been found to be both dependent and independent of changes in γ -secretase activity [1,20]. Multiple factors might explain these differences including cell-type studied, protocols used to elicit responses, and the specific PS1 constructs or drugs used to inhibit γ -secretase activity. PS1 mutations influence multiple cell signaling pathways including those regulated by phospholipase C [4], glycogen synthase kinase [15] and mitogen-activated protein kinase [16]. The multifunctional calcium binding protein, calsenilin, binds PS proteins and participates in Ca^{2+} -dependent gene expression. Expression of mutant presenilins might also disrupt ER structure altering ER-SOCE coupling. However, experiments with DAPT indicate that inhibition of proteolytic activity produces the increase in SOCE. Interestingly, acute application of DAPT had no effect. Thus, the most parsimonious explanation for the up regulation of SOCE described here is a change in transcription resulting from γ -secretase inhibition.

Presumably the SOCE observed in cells expressing PS1-D257A is also present in non-transfected cells, although less prominent. Thus, PS1-D257A may be used to enhance SOCE to enable its study in sensory neurons. CPA-induced Ca^{2+} entry in cells expressing PS1-D257A was inhibited by SKF96365 consistent with the pharmacology of SOCE [22], although truly selective blockers for these channels are not available. In DAPT-treated cells, SKF96365 produced complicated effects on the CPA-induced $[Ca^{2+}]_i$ response that included a slowly developing increase in $[Ca^{2+}]_i$. In some cells, SKF96365 activates a non-selective cation channel at high concentrations [22]. The appearance of this Ca^{2+} influx response in DAPT-treated, but not PS1-D257A-expressing cells, suggests that inhibition of γ -secretase is not sufficient to reveal this effect of SKF96365 in DRG neurons.

The modulation of SOCE by presenilin-dependent proteolytic activity most likely results from altered expression of the store operated channel or its regulator. We speculate that release of the intracellular signaling domain following proteolytic cleavage of a substrate such as Notch results in transcription of genes encoding channel or regulatory proteins such as STIM1, Orai and TRPC. Expression of a dominant-negative PS1 mutant in HEK cells enhanced agonist-induced Ca^{2+} influx through TRPC6 channels suggesting that PS might control an intermediate protein, either via transcription or direct binding, which then regulates channel activity [21].

TRPC channels participate in SOCE [24] and several TRPC channel isoforms are expressed in DRG neurons [18].

The role for SOCE and PS1 in sensory physiology is unclear at present although the dominant negative PS1 mutant should prove useful for studying these signaling pathways. The altered channel function described here suggests that SOCE and presenilins might cooperate to produce long-term changes in neuronal excitability.

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Fig. 1.

PS1-D257A expression inhibits γ -secretase and prolongs SOCE in DRG neurons. (A) Expression of PS1-D257A inhibits CBF1-dependent transcription. Cultures were transfected with 4xwtCBF1Luc and pRL-CMV. Luciferase activity, expressed as the ratio of firefly to *Renilla* luminescence, was measured from populations of cells expressing EGFP alone (Sham) or EGFP and either PS1-D257A or PS1-WT. * $p < 0.05$ PS1-D257A versus PS1-WT or sham. (B) Representative trace from a cell expressing PS1-D257A shows protocol for SOCE evoked by continuous exposure to 5 μ M CPA. CPA was applied during the time indicated by the horizontal bar. (C) Bar graph depicts mean $[Ca^{2+}]_i$ calculated for the variables listed. The letters correspond to the times indicated in B. * $p < 0.05$, compared to Sham or PS1-WT. $p = 0.06$, PS1-D257A compared to PS1-WT (D) Representative SOCE traces for Sham ($n = 28$), PS1-D257A ($n = 34$), and PS1-WT ($n = 14$) expressing neurons are shown. (E) Time to recover to 75% of the peak SOCE response is shown for sham, PS1-D257A and PS1-WT expressing neurons. * $p < 0.05$, compared to Sham or PS1-WT.

Fig. 2. PS1-D257A expression in DRG neurons prolongs SOCE. (A) Representative trace showing protocol used to study SOCE in rat DRG neurons. Treatments were applied at the times indicated by the horizontal bars. Cells were bathed in 1.26 mM Ca^{2+} except where indicated. The boxed region contains the SOCE response induced by returning Ca^{2+} to the extracellular solution. (B) Representative SOCE traces from Sham (n=6) and PS1-D257A (n=6) expressing cells are shown for the time indicated by the boxed region in A. (C) Bar graph summarizes the effects of PS1-D257A expression on $[Ca^{2+}]_i$ for PS1-D257A and Sham transfected cells at the times listed. The letters in parentheses correspond to the times indicated in A. *p<0.05 versus Sham. (D) Bar graph shows time to recover to 75 % of the peak SOCE in Sham and PS1-D257A expressing neurons. *p<0.05, PS1-D257A versus sham.

Fig. 3.

DAPT prolongs SOCE in DRG neurons. Neurons were treated in culture with the indicated concentration of DAPT or DMSO (control) for 48 hours. Cells were washed for 15 minutes before beginning the recording. Both wash and recording periods are in the absence of vehicle or drug. (A) DAPT inhibited transactivation of CBF1 luciferase. * $p < 0.05$, 100 μM DAPT compared to sham. (B) Representative $[\text{Ca}^{2+}]_i$ traces for control neurons ($n=9$) and neurons treated with 100 μM DAPT ($n=17$) were normalized to the peak $[\text{Ca}^{2+}]_i$ and overlaid. (C) Bar graph summarizes the effects of 48 hr treatment with 100 μM DAPT on the $[\text{Ca}^{2+}]_i$ measured after 2000 s in CPA (5 μM). * $p < 0.05$, DAPT-treated (100 μM) compared to control.

Fig. 4.

SKF96365 inhibits SOCE in DRG neurons. (A-B) Representative traces show SOCE evoked by 5 μ M CPA (applied at the times indicated by the horizontal bars) in PS1-D257A expressing cells (A) and in cells treated for 48 hrs with 100 μ M DAPT (B) in the absence or presence of 10 μ M SKF96365 (added 5 min prior to and during CPA treatment). (C) Bar graph summarizes the effects of 10 μ M SKF96365 on the $[Ca^{2+}]_i$ measured after 2000 s in CPA from untreated (control), PS1-D257A expressing and DAPT (48 hrs) treated cells ($n \geq 5$ for each group).

* $p < 0.05$, compared to vehicle-treated cells.