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Presenilin-1 mutations associated with familial Alzheimer's disease do not disrupt protein transport from the endoplasmic reticulum to the Golgi apparatus

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Abstract

Mutations in genes encoding presenilin-1 (PS1) and presenilin-2 (PS2) have been linked to familial forms of Alzheimer's disease (AD). Cells expressing mutant presenilins produce elevated levels of $A\beta_{42}$, the major amyloid peptide found in AD plaques. The mechanism whereby this occurs remains unknown, but the localization of presenilins to endoplasmic reticulum (ER) and Golgi compartments has suggested that they may function in intracellular trafficking pathways involved in processing β-amyloid precursor proteins (APP). To test this possibility, we coexpressed PS1(wt), PS1(M146L), or PS1(L286V) in HEK293 cells together with the LDL receptor, a classic glycoprotein marker that undergoes post-translational O-glycosylation in the Golgi compartment. Pulse-chase analysis of the receptor indicated that mutant presenilins had no effect on ER → Golgi transport. Similar results were obtained when the studies were carried out with cells expressing the Swedish variant of APP (SWAPP$_{751}$) instead of the LDL receptor. Moreover, secretion of the soluble exodomain polypeptide fragments of SWAPP$_{751}$ that arise from α-secretase and β-secretase cleavage was not markedly affected by the PS1 mutants. Despite the lack of discernible effect of the PS1 mutants on trafficking of proteins through the Golgi apparatus, they caused a substantial increase in the proportion of $A\beta_{42}$ relative to total Aβ in the culture medium. The results suggest that mutant forms of PS1 cause elevated production of $A\beta_{42}$ by a mechanism that is independent of a major disruption of exocytic trafficking of APP.

Keywords: Presenilin; Alzheimer's disease; Protein trafficking; β-Amyloid precursor protein; Amyloid β-peptide; Endoplasmic reticulum; Golgi

1. Introduction

Amyloid β-peptide (Aβ) is a major component of cerebral amyloid plaques in Alzheimer's disease (AD). Aβ is formed in neurons and other cell types through proteolytic processing of a membrane-anchored glycoprotein precursor; β-amyloid precursor protein (APP) [1–3]. Processing of APP can occur via at least two alternative routes: the bulk of APP enters a non-amyloidogenic pathway in which the precursor is first cleaved within the Aβ domain by a protease termed α-secretase, leaving a C-terminal fragment that is subsequently cleaved by γ-secretase. In the alternative amyloidogenic pathway, APP is

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initially cleaved proximal to the Aβ sequence by an activity termed β-secretase, leaving a C-terminal fragment that can give rise to Aβ when it is finally cleaved by γ-secretase. It is now well established that cells can produce alternate forms of Aβ that differ in overall chain length (e.g. $A\beta_{40}$ and $A\beta_{42}$), with the longer forms being the most prone to form insoluble amyloid deposits [4–6]. Moreover, recent evidence suggests that the different forms of Aβ may be generated through the action of distinct γ-secretase activities [7,8].

Some early-onset familial forms of AD are known to be associated with mutations in APP which result in an increased proportion of the protein being processed via the amyloidogenic route [9–12]. More recently, mutations in a new class of genes encoding proteins termed presenilin-1 (PS1) [13] and presenilin-2 (PS2) [14] have also been linked to familial AD. In particular, mutations in PS1 appear to account for the majority of cases of early-onset familial AD [15,16]. PS1 and PS2 are polypeptides of 467 and 448 amino acids, respectively, that are predicted to have serpentine structures with multiple hydrophobic membrane-spanning segments [13,17,18]. Although the biological functions of the presenilins are not yet known, recent evidence suggests that mutations in these proteins may somehow alter the processing of APP in manner that favors increased production of $A\beta_{42}$ [19–22].

Presenilins show significant similarity to two proteins in *Caenorhabditis elegans*; i.e. Sel-12 [23] and Spe-4 [24]. Spe-4 appears to be involved in the formation of a specialized Golgi-derived structure termed the fibrous body membrane organelle during spermatogenesis. Sel-12 facilitates signaling though the lin-12/Notch family of receptors, possibly through effects on localization or recycling of the receptors. Interestingly, expression of PS1 can compensate for defects in *sel-12* in *C. elegans*, suggesting functional homology between Sel-12 and the presenilins [25]. The foregoing observations, coupled with immunolocalization studies indicating that PS1 is distributed primarily in the endoplasmic reticulum (ER) and Golgi apparatus [26–30], have raised the intriguing possibility that presenilins could be components of the intracellular machinery for vesicular transport and protein trafficking. If true, this could have important implications for the mechanism whereby presenilin mutants increase production of $A\beta_{42}$. For instance, recent reports have provided evidence that, unlike $A\beta_{40}$, $A\beta_{42}$ may be generated predominantly within the ER [31,32]. Thus, one can envision a mechanism wherein impairment of normal ER → Golgi trafficking of APP by mutant presenilins might increase the biogenesis of $A\beta_{42}$ by allowing nascent APP to be retained in an early subcellular compartment containing the $A\beta_{42}$-forming γ-secretase activity.

In the present study, we have tested the hypothesis that mutant forms of PS1 implicated in familial AD produce elevated levels of $A\beta_{42}$ through a disruption of ER → Golgi protein trafficking. This was done by assessing the effects of wild-type and mutant forms of PS1 on the post-translational processing of a model glycoprotein, the human low density lipoprotein (LDL) receptor, as well as the Swedish variant of APP in transfected human 293 cells.

2. Materials and methods

2.1. Expression vectors

The pohCk751sw mammalian expression vector has been described previously [33]. It encodes the Swedish variant of APP_{751} which contains a dual amino acid change ($K_{651} \rightarrow N$; $M_{652} \rightarrow L$) that increases the susceptibility of the protein to amyloidogenic processing [10–12]. The plasmid, pLDLR17 which contains the full-length sequence encoding the human LDL receptor inserted into pCMV4 [34], was provided by David Russell, University of Texas Southwestern Medical Center. The cDNA encoding human PS1 was obtained by polymerase chain reaction (PCR) amplification as follows: Total cDNA was synthesized from human brain poly(A)$^+$ RNA (Clontech, Palo Alto, CA) using SuperScript II reverse transcriptase (Gibco, Gaithersburg, MD) and oligo-dT primer. The brain cDNA was then used as template in a PCR reaction containing a 20:1 ratio of AmpliTaq DNA polymerase and ULTma DNA polymerase (Perkin–Elmer, Foster City, CA) and primers flanking the PS1 sequence (5′-CAGGGTCGCGATGACAGAGTTACCTGCACCGTTGTCCTAC and 5′-GCCTGACTAGTCTAGATATAAAATTGATG-GAATGCTAATTG). The first-round

product obtained after 30 cycles (0.5 min at 94°C, 1 min at 57°C and 5 min at 72°C) was used as a template in a second-round PCR reaction under the same conditions to obtain sufficient material for cloning. The final PS1 product was cloned into pNoTA/T7 (5 Prime→3 Prime, Boulder, CO) and the DNA sequence was determined. The individual mutations L286→V and M146→L were created in the recombinant plasmid, pNoTA/T7(PS1), using the Transformer site-directed mutagenesis kit as directed by the manufacturer (Clontech, Palo Alto, CA). The wild-type and mutant PS1 genes were then subcloned into the mammalian expression vector, pohCk751 [33], using the *Nru*I and *Spe*I restriction sites.

2.2. Transfection of cultured cells

Transformed human embryonal kidney cells (line 293), obtained from American Type Culture Collection, were maintained at 37°C in a 5% CO_2 atmosphere in Dulbecco's modified Eagle's medium (DMEM) containing 10% (v/v) fetal bovine serum (FBS). Cells were plated in 60-mm dishes at 7.5×10^5 cells/dish on the day before transfection. In studies aimed at assessing the effects of PS1 mutants on processing of the LDL receptor, parallel cultures were co-transfected with pLDLR17 in combination with either pohCkPS1(wt), pohCkPS1(M146L), or pohCkPS1(L286V), using Superfect transfection reagent according to the protocol recommended by the manufacturer (Qiagen). In separate studies designed to evaluate the effects of PS1 mutants on APP processing, the same approach was used, except that the PS1 constructs were co-transfected with pohCk751sw instead of pLDLR17.

2.3. Metabolic labeling and immunoprecipitation of LDL receptor or APP

Twenty-four hours after transfection, cultures were pulse-labeled for 30 min at 37°C with 1 ml methionine-free DMEM containing 100 μCi of [^{35}S]methionine/cysteine (Trans-label, 1100–1200 Ci/mmol, ICN). The cells were then washed twice with PBS and subjected to a 2 h chase in DMEM containing 10% FBS, 2 mM methionine, and 2 mM cysteine. Cells were harvested from parallel cultures at the end of the pulse and chase periods and cell extracts were prepared by disrupting the cells in phosphate-buffered saline solution (PBS) containing 1% (v/v) Triton X-100. The lysates were cleared by centrifugation at $10\,000\times g$ for 10 min at 4°C, and one-tenth of the supernatant solution was removed for assessment of protein expression levels by immunoblot analysis. The remainder of the sample was used for immunoprecipitation of radiolabeled LDL receptor [35] or APP [33] as described previously. Precipitated proteins were subjected to SDS-PAGE and autoradiography and the radioactivity incorporated into the processed versus unprocessed forms of LDL receptor or APP was quantified using a Molecular Dynamics phosphorimager and ImageQuant software.

2.4. Immunoblot analysis

Aliquots of cell lysates or conditioned culture medium were mixed with 2×SDS sample buffer so that the final composition was 8 M urea, 2% w/v SDS, 5% v/v 2-mercaptoethanol, 10% v/v glycerol, 62.5 mM Tris-HCl, pH 6.8. Proteins were resolved by SDS-PAGE, using 10% polyacrylamide gels for analysis of PS1 and 6.5% polyacrylamide gels for analysis of the LDL receptor or APP. Proteins were transferred to Immobilon-P (Millipore) and the membranes were preincubated for 1 h in blotting solution (PBS containing 5% w/v powdered milk and 0.1% v/v Tween 20). PS1 was detected with a polyclonal antibody (199) raised against a KLH-coupled peptide corresponding to amino acids 333–347 in the large cytoplasmic loop of PS1. Intracellular APP_{751} and total secreted forms of APP (s-APP_{total}) were detected with monoclonal antibody 8E5 [36]. The secreted exodomain fragment released from *SW*APP_{751} as a result of β-secretase cleavage (s-APPβ) was detected with a polyclonal antibody, SW192 [37]. All primary antibodies were used at a final concentration of 0.1 μg/ml, and immunoblotting procedures were carried out as described previously [33,38]. For chemiluminescent detection with the ECL kit (Amersham), horseradish peroxidase-conjugated goat anti-rabbit IgG or goat anti-mouse IgG were obtained from Bio-Rad and used at 1:3000 v/v dilution. For quantitative immunoblot analysis of intracellular and secreted forms of APP, ^{125}I-labeled goat anti-mouse IgG or goat anti-rabbit IgG were obtained from Du-

Pont/NEN and used at a final concentration of 0.45 μCi/ml. Bound [^{125}I]IgG was detected by autoradiography and quantified by phosphorimager analysis.

2.5. ELISA for Aβ

Conditioned medium (3 ml total) was collected from each culture between 24–48 h after transfection. Aliquots of medium were preadsorbed with heparin-agarose and the concentrations of total Aβ or Aβ$_{42}$ were determined by sandwich-type ELISA, as described previously [38].

3. Results

3.1. Effect of PS1 mutants on Golgi-dependent processing of the human LDL receptor

The nascent human LDL receptor is modified by high-mannose N-linked carbohydrate chains in the ER and exhibits an apparent molecular mass of 120 kDa when subjected to SDS gel electrophoresis. Upon its translocation to the Golgi apparatus, the protein is converted to a mature form which migrates at approximately 160 kDa [39]. The shift in electrophoretic mobility reflects the addition of O-linked oligosaccharide chains, and to a lesser extent, the trimming and sialylation of the N-linked carbohydrates [40]. Thus, the change in mobility of the LDL receptor provides a convenient means to monitor glycoprotein trafficking from the ER to the Golgi compartment. For example, in a previous study, we showed that disruption of ER→Golgi vesicular transport by expression of a dominant-negative Rab1B GTPase mutant in 293 cells prevents the conversion of nascent LDL receptor to the mature high-molecular mass form [35].

To examine the possibility that mutant forms of PS1 might cause elevated levels of Aβ$_{42}$ through a general perturbation of ER→Golgi protein transport, we initially tested the effects of wild-type and mutant forms of PS1 on the maturation of the LDL receptor. HEK293 cells were co-transfected with expression vectors encoding the human LDL receptor, either alone or in combination with vectors encoding PS1(wt), PS1(M146L), or PS1(L286V). Twenty four hours after transfection, the cells were pulse-labeled

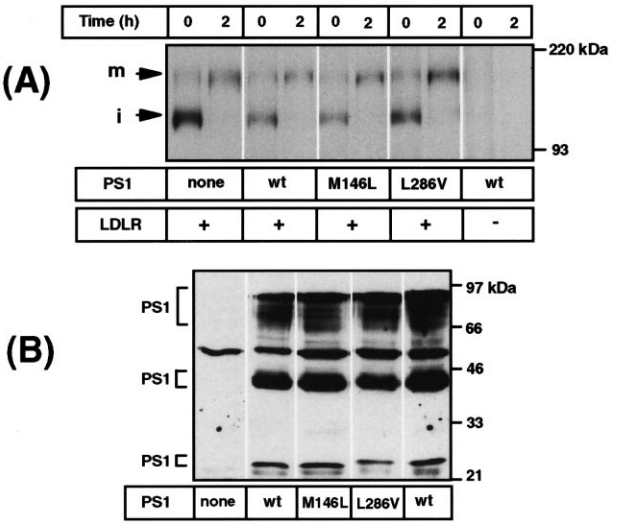

Fig. 1. Effects of wild-type or mutant PS1 on post-translational maturation of the human LDL receptor expressed in 293 cells. (A) Parallel cultures of human HEK293 cells were co-transfected with expression vectors encoding the LDL receptor combined with empty vector (none) or vector encoding either PS1(wt), PS1(M146L), or PS1(L286V) as indicated. Twenty four hours after transfection the medium was changed and cells were pulse-labeled with [^{35}S]methionine for 30 min, then subjected to a 2 h chase as described in Section 2. Cells were harvested immediately after pulse-labeling (0 h) or after the chase (2 h) as indicated and the immunoprecipitated LDL receptor was subjected to SDS-PAGE and fluorography. The immature (i) and mature (m) forms of the receptor are indicated by the arrows at the left of the figure. (B) Immunoblot analyses were performed on aliquots of the cell lysates from the cultures harvested at the end of the chase period to confirm expression of PS1 constructs, using a polyclonal antibody against the large cytoplasmic loop of PS1 as described in Section 2. Specific bands corresponding to overexpressed PS1 are indicated on the left of the figure. Results similar to those described in this figure were obtained in two additional experiments.

with [^{35}S]methionine and maturation of the LDL receptor was assessed by electrophoretic analysis of the immunoprecipitated protein. As shown in Fig. 1A, no radiolabeled LDL receptor was detected in 293 cells that received the PS1(wt) plasmid alone, signifying that background expression of endogenous LDL receptor was extremely low. Cells that received the LDL receptor plasmid alone showed efficient ER→Golgi dependent processing of the expressed protein, with the 120 kDa precursor form of the receptor predominating immediately after the pulse-labeling period and the 160 kDa mature form of the protein predominating at the end of the 2-h chase.

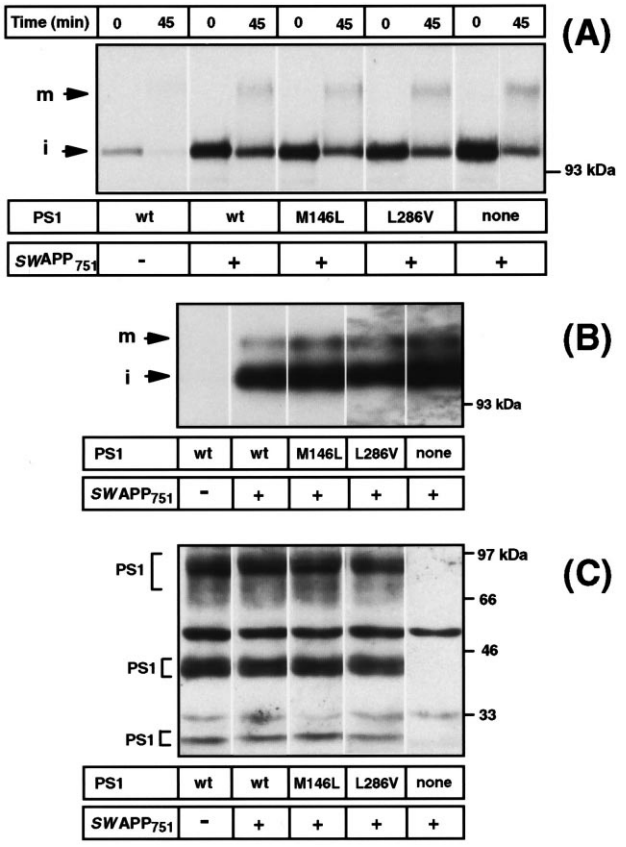

Fig. 2. Effects of wild-type or mutant PS1 on post-translational maturation of *SW*APP$_{751}$ in 293 cells. (A) Parallel cultures of human HEK293 cells were co-transfected with expression vectors encoding *SW*APP$_{751}$ combined with empty vector (none) or vector encoding either PS1(wt), PS1(M146L), or PS1(L286V) as indicated. Twenty four hours after transfection the medium was changed and cells were pulse-labeled with [^{35}S]methionine for 10 min, then subjected to a 45 min chase as described in Section 2. Cells were harvested immediately after pulse-labeling (0 min) or after the chase (45 min) as indicated, and the immunoprecipitated APP was subjected to SDS-PAGE and fluorography. The immature (i) and mature (m) forms of APP are indicated by the arrows at the left of the figure. (B) Immunoblot analyses were performed on aliquots of the cell lysates from the cultures harvested at the end of the chase period to determine the steady-state levels of immature and mature intracellular *SW*APP$_{751}$, using a monoclonal antibody that recognizes all forms of APP. (C) Immunoblot analyses were performed on aliquots of the cell lysates from the cultures harvested at the end of the chase period to confirm expression of the presenilin constructs as described in Section 2. Specific bands corresponding to overexpressed PS1 are indicated on the left of the figure.

(Fig. 1A). In parallel cultures where the LDL receptor was coexpressed with PS1(wt), PS1(M146L), or PS1(L286V), the results were essentially the same as in the cultures where the receptor was expressed without PS1; i.e. nearly all of the nascent receptor was converted to the mature 160 kDa form by the end of the chase (Fig. 1A). This contrasts markedly with the readily detected perturbation of LDL receptor maturation observed in our previous studies where ER→Golgi transport was blocked by expression of a dominant-negative mutant form of the Rab1B GTPase in 293 cells [35].

To verify that PS1 proteins were indeed expressed in the transfected cells, aliquots of the cell lysates used for immunoprecipitation of the LDL receptor were subjected to immunoblot analysis using a polyclonal antibody against PS1 (Fig. 1B). Consistent with previous studies [26,41–43], full-length PS1, which migrates at approximately 42 kDa, was readily detected in cells that were transfected with constructs encoding PS1(wt), PS1(M146L), or PS1(L286V), but not in cells that received the LDL receptor plasmid alone. All of the cultures expressing PS1 also contained substantial amounts of immunoreactive protein in the high molecular weight region of the blots. This material is thought to represent aggregates of the overexpressed PS1 [29,30,42,44]. In addition to the full-length and aggregated forms of PS1, we also observed a specific immunoreactive band at approximately 23 kDa, which may correspond to a PS1 carboxyl-terminal proteolytic fragment similar to that described by others [41,43,45]. Confirmation of the identity of the immunoreactive proteins was obtained by adding excess peptide corresponding to amino acids 333–397 of PS1 during the incubation with the primary antibody. Under these conditions, the bands at 66–95, 42 and 23 kDa were not detected, whereas the apparent cross-reacting protein band at 53 kDa remained (data not shown). The expression levels of all of the electrophoretic forms of PS1 were comparable in the cells expressing either the wild-type or mutant PS1 constructs, consistent with a report indicating that most FAD point mutations do not affect proteolytic processing of PS1 [46].

3.2. *Effects of PS1 mutants on ER→Golgi trafficking of SWAPP$_{751}$ in 293 cells*

Like the LDL receptor, the Alzheimer's β-amyloid precursor protein (APP) undergoes a shift in electrophoretic mobility after it is O-glycosylated and sul-

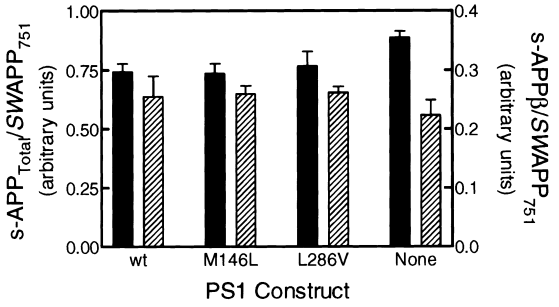

Fig. 3. Effects of wild-type or mutant PS1 on steady-state concentrations of soluble APP derivatives secreted into the culture medium. Cells were co-transfected with expression vectors encoding *SW*APP$_{751}$ combined with empty vector (none) or vector encoding either PS1(wt), PS1(M146L), or PS1(L286V) as indicated. Cells and medium were collected 48 h after transfection. Immunoblot assays were performed on aliquots of the culture medium, using monoclonal antibody 8E5, which detects both s-APPα and s-APPβ (s-APP$_{Total}$) (solid bars) or polyclonal antibody SW192, which detects only s-APPβ derived from the Swedish variant of APP (cross-hatched bars). In each case, the bound secondary [^{125}I]IgG was quantified by densitometric scanning of autoradiograms as described in Section 2. Each value for s-APP (in arbitrary densitometer units) was expressed as a ratio to the total intracellular *SW*APP$_{751}$ (arbitrary units) detected by immunoblot assay of the corresponding cell monolayer using the 8E5 monoclonal antibody. Each bar represents the mean ± S.E. derived from separate determinations on three parallel cultures.

fated in the medial-late Golgi compartment [47,48]. In a previous study, we determined that when ER → Golgi transport is blocked in 293 cells, either by treatment with brefeldin A or by overexpression of a dominant-negative Rab1B mutant, the post-translational maturation of APP is severely impaired [33]. Therefore, to further evaluate the possible relationship between mutations in PS1 and ER → Golgi protein transport, we coexpressed PS(wt), PS1(M146L) or PS1(L286V) with the Swedish variant of APP (*SW*APP$_{751}$) and examined the post-translational processing of metabolically labeled APP by pulse-chase analysis on the day after transfection (Fig. 2A). As in the studies of the LDL receptor, the results of this experiment did not reveal any obvious inhibitory effects of the PS1 mutants on the conversion of the immature ER form of *SW*APP$_{751}$ (approximately 108 kDa) to the mature form (approximately 135 kDa). In agreement with these findings, the steady-state amounts of mature versus immature *SW*APP$_{751}$ detected by immunoblot analysis were not noticeably different in cells expressing wild-type or mutant PS1 compared to cells expressing no exogenous PS1 (Fig. 2B). Finally, as in the previous experiments with the LDL receptor, expression of PS1 was confirmed in all of the cultures transfected with PS1 vectors by the appearance of immunodetectable bands corresponding to full-length (42 kDa), aggregated (66–95 kDa), and proteolytically cleaved (23 kDa) forms of PS1 in the transfected cell cultures (Fig. 2C).

3.3. *Effects of PS1 mutants on secreted products derived from SWAPP$_{751}$ in 293 cells*

Cleavage of APP by α-secretase is thought to occur in a late compartment of the constitutive secretory pathway [49–52]. Studies of the Swedish variant of APP have suggested that the alternative β-secretase cleavage occurs in an earlier Golgi compartment [38,53,54]. In both cases the resulting soluble amino-terminal exodomain fragments (s-APPα or s-APPβ) are subsequently secreted into the extracellular me-

Table 1
Effects of wild-type or mutant PS1 on the production of amyloid β-peptides in 293 cell cultures expressing the Swedish variant of APP$_{751}$

PS1 construct	Aβ$_{42}$ (pg/culture)	Aβ$_{Total}$ (pg/culture)	Aβ$_{42}$ (% of total Aβ)	% Increase in Aβ$_{42}$
None	64 ± 10	2970 ± 399	2.15 ± 0.04	
wt	69 ± 6	3371 ± 312	2.05 ± 0.01	−5
M146L	119 ± 5	3798 ± 110	3.13 ± 0.02	46
L286V	77 ± 6	2741 ± 166	2.80 ± 0.06	30

The concentrations of total Aβ (predominantly Aβ$_{40}$) or Aβ$_{42}$ were determined in aliquots of conditioned culture medium from the experiment described in Fig. 3 using an established ELISA (see Section 2). Each value is a mean ± S.E. of separate determinations performed on three parallel cultures. No Aβ$_{42}$ was detected in the medium from the control 293 cells that were not transfected with the plasmid encoding *SW*APP751$_{751}$.

dium. To determine whether mutations in PS1 might disrupt intracellular trafficking of *SW*APP at points distal to the initial ER→Golgi step, we performed immunoblot analyses of conditioned medium to compare the relative concentrations of total s-APP (α+β forms) or s-APPβ released from 293 cells that had been co-transfected with *SW*APP_{751} and various PS1 constructs. The results depicted in Fig. 3 indicate that neither the wild-type nor the mutant PS1 constructs had any significant cumulative effect on the secretion of soluble forms of APP into the culture medium, implying that the α and β secretase processing steps were unimpaired.

Finally, we wished to verify that under the conditions employed in the foregoing studies, the mutant forms of PS1 did indeed cause an increase in cellular production of $Aβ_{42}$ relative to $Aβ_{40}$ as reported previously [20]. Thus, samples of conditioned medium from cultures that were expressing *SW*APP_{751} in the presence or absence of PS1 were subjected to ELISAs for total Aβ and $Aβ_{42}$, and the ratio of $Aβ_{42}/Aβ_{Total}$ was determined (Table 1). The results confirmed that the relative amounts of $Aβ_{42}$ produced by the cells expressing PS1(M146L) or PS1(L286V) were substantially elevated compared to cells that were expressing PS1(wt) or no exogenous PS1.

4. Discussion

The results demonstrate that under conditions where expression of mutant forms of PS1 in 293 cells produce a significant elevation of $Aβ_{42}$ deposition into the culture medium, there are no major disruptions of ER→Golgi trafficking and secretory processing of APP or an unrelated intracellular glycoprotein, the LDL receptor. Previous studies have shown that such disruptions are readily detected by the pulse-chase assays employed in the present studies [33,35]. In recent years there has been much progress in defining the molecular events underlying the packaging of ER-derived cargo proteins into transport vesicles and their subsequent export to the Golgi stack (for recent reviews see [55,56]). Increasing evidence suggests that this is a selective process, with different proteins being sorted into budding vesicles and transported at different rates. However, all normally folded nascent protein molecules of a particular type (for example, APP_{751}) are expected to behave as a homogeneous pool as they are concentrated in vesicles and exported to the Golgi compartment. Based on these considerations, we conclude that mutations in PS1 do not cause increased $Aβ_{42}$ formation by impairing the normal flow of APP out of the ER into the constitutive secretory pathway.

It remains possible that $Aβ_{42}$ is derived from a small pool of APP that becomes misfolded or otherwise modified in the ER so that it fails to enter the constitutive ER→Golgi export pathway. This minor pool of APP could be subject to alternative trafficking within specialized subdomains of the ER [57] or perhaps between the ER and components of the endosomal/lysosomal system [58]. Perturbations in such pathways, possibly mediated by presenilin mutants, would not be detected by conventional pulse-chase analysis of Golgi-dependent protein processing. In addition, our studies do not rule out the possibility that mutant forms of PS1 could increase the biogenesis of $Aβ_{42}$ by altering the internalization of APP from the cell surface or the trafficking of APP through the endocytic pathway [59,60]. However, this possibility seems remote in light of multiple reports documenting the localization of PS1 in ER/Golgi membranes [26–30] and evidence that long forms of Aβ are generated predominantly in the latter compartments [31,32]. A final caveat that must be considered in the interpretation of any studies where presenilin is overexpressed in transfected cells is that the properties of the overexpressed protein may not completely reflect those of the endogenous protein. Of particular note is the fact that much of the overexpressed PS1 tends to accumulate in a full-length form, whereas endogenous PS1 appears to be efficiently processed into N-terminal and C-terminal fragments by an undefined proteolytic pathway [41,43]. Moreover, there is some evidence that alternative pathways for proteolytic processing of PS1 may occur in differentiated neurons [61,62], and that the protein may be present at subcellular sites beyond the ER in such cells [26,63].

If presenilins do not participate directly in the trafficking of APP, then other mechanisms must be invoked to explain the ability of mutant presenilins to affect $Aβ_{42}$ production. In this regard, recent reports have uncovered several intriguing leads for future exploration. For example, the co-immunoprecipita-

tion studies of Xia et al. [45] indicate that presenilins can associate directly with immature forms of APP that reside in the ER, raising the possibility that such interactions might affect the susceptibility of APP to proteolytic processing by a specific γ-secretase responsible for generating $A\beta_{42}$, but not $A\beta_{40}$ [7,8]. Alternatively, one may envision mechanisms whereby mutant presenilins could alter Aβ production through indirect effects on other metabolic pathways. For instance, Guo et al. [64] have shown that PC12 cells expressing PS1(L286V) are more sensitive to apoptotic stimuli than cells expressing PS1(wt), and that this effect may be related to changes in intracellular calcium homeostasis. Along an entirely different line, the recent demonstration of presenilins in the nuclear membrane and centrosomes suggests a role for these proteins in chromatin organization and/or chromosome segregation, distinct from their function in APP processing [65]. The latter view would be consistent with the finding that PS1-deficient mice exhibit major defects in neurogenesis and development of the axial skeleton [66]. Insofar as the present studies argue against a direct role for presenilins in the constitutive exocytic vesicular transport machinery, they should help to narrow the focus of future studies aimed at defining the biological functions of these intriguing proteins.

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