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Proteasome Inhibitors Prevent the Degradation of Familial Alzheimer's Disease-Linked Presenilin 1 and Potentiate Aβ42 Recovery from Human Cells

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Abstract

Background: Several lines of evidence suggest that most of the early-onset forms of familial Alzheimer's disease (FAD) are due to inherited mutations borne by a chromosome 14–encoded protein, presenilin 1 (PS1). This is likely related to an increased production of amyloid β-peptide (Aβ)42, one of the main components of the extracellular deposits called senile plaques that invade human cortical areas during the disease.

Materials and Methods: We set up stably transfected HEK293 cells overexpressing wild-type (wt) and various FAD-linked mutated PS1. By Western blot analysis, we examined the influence of specific proteasome inhibitors on PS1-like immunoreactivities. Furthermore, by means of metabolic labeling and immunoprecipitation with Aβ40 and Aβ42-directed specific antibodies, we assessed the effect of the inhibitors on the production of Aβs by wt and mutated PS1-expressing cells transiently transfected with βAPP751.

Results: We show that two distinct proteasome inhibitors, Z-IE(Ot-Bu)A-Leucinal and lactacystin, increase in a time- and dose-dependent manner the immunoreactivities of both wt and mutated PS1. Furthermore, we demonstrate that PS1 is polyubiquitinated in these cells. Other inhibitors, ineffective on the proteasome, fail to protect wt and mutated PS1-like immunoreactivities. We also establish that the FAD-linked mutations of PS1 trigger a selective increased formation of Aβ42 as reflected by higher Aβ42 over total Aβ ratios when compared with wtPS1-expressing cells. Interestingly, this augmentation was further amplified by proteasome inhibitors in cells expressing mutated but not wtPS1.

Conclusion: Altogether, our data indicate that PS1 undergoes polyubiquitination in HEK293 cells and that the proteasome contributes to the degradation of wt and FAD-linked PS1, thereby directly influencing the Aβ production in human cells.

Introduction

A network of independent studies has led to the suggestion that the presenilins (PS) likely contribute to the physiopathological maturation of the β amyloid precursor protein (βAPP). Thus, consensual data indicate that mutations on PS, which are responsible for agressive early-onset forms of Alzheimer's disease (AD), consistently lead to increased formation of the pathogenic amyloid β-peptide (Aβ)42 species. This was not only shown in the brains of affected patients (1–3) but also evidenced by means of transfected cells (4–7) and transgenic animal models

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(4,5,6,8). Interestingly, we recently showed that mutations on PS1 could also trigger decreased secretion of the physiological secreted product APPα (9). Although it is not yet demonstrated, it can be postulated that PS likely interfere with or control the routing of βAPP, as these two proteins often colocalize in the central nervous system (10–12) and are able to physically interact (13,14).

We previously established that the proteasome contributes to the α-secretase pathway. Thus, two proteasome inhibitors, lactacystin and Z-IE(Ot-Bu)A-Leucinal, increased constitutive APPα secretion by human cells. Furthermore, the proteasome appears to exert a dual control over protein kinase C (PKC)-regulated APPα secretion by human kidney (HEK)293 cells, as short-term treatment of these cells with proteasome inhibitors led to enhanced recovery of APPα whereas prolonged exposure of the cells triggered decreased APPα recovery (15,16). We established that PKC effectors modified neither the proteasome activity nor its basal phosphorylation state (17) in vitro and in vivo. This indicates that the proteasome does not correspond to α-secretase itself, and it suggests the likely occurrence of another intermediate involved in βAPP processing that should behave as a substrate of this multicatalytic complex.

We present here evidence that wild-type and familial Alzheimer's disease (FAD)-linked PS1 behave as substrates of the proteasome in stably transfected HEK293 cells. Furthermore, we demonstrate that proteasome inhibitors modulate the production of Aβ secretion by these cells, and particularly, that they exacerbate the phenotypic overproduction of Aβ42 by cells overexpressing the mutated forms of PS1. Combined with our previous studies, these data suggest that the proteasome could control the intracellular levels of PS1, upstream to PS1 and βAPP interaction, thereby influencing the production of physiological and pathogenic catabolites of βAPP maturation.

Materials and Methods

Molecular Cloning of PS1 and Mutagenesis

PS1 was cloned as previously described (18). A polymerase chain reaction (PCR)-derived probe was obtained from a human kidney cDNA library constructed in the λZAPII vector. This PCR probe was used to screen the above cDNA library, leading to the isolation of four PS1 cDNA clones, the sequences of which correspond to full-length PS1. The Met146Val, His163Arg, and Glu280Ala mutations (18) and the ΔE9-PS1 cDNA (19) were obtained as previously documented.

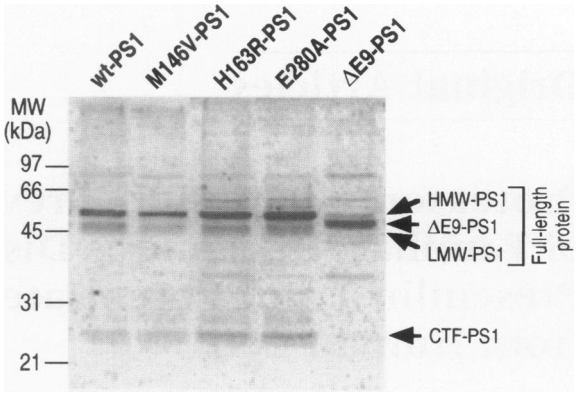

Fig. 1. Wild-type and FAD-linked PS1 expression in stably transfected HEK293 cells. HEK293 cells were stably transfected with pcDNA3 containing wild-type PS1, ΔE9-PS1, or indicated missense mutated PS1. Transfected cells were grown in the presence of geneticin, then scraped, lysed in TBS-SDS, and 20 μg of protein was electrophoresed on a 12% SDS-PAGE analysis. PS1 holoproteins of high molecular weight (HMW-PS1), low molecular weight (LMW-PS1), and corresponding carboxyl-terminal fragment (CTF-PS1) were then revealed with αPS1Loop antibody as described in Materials and Methods.

Stable Transfections in HEK293 Cells

HEK293 cells were stably transfected by calcium phosphate precipitation with 1 μg of pcDNA3 containing either wild-type (wt) PS1, ΔE9-PS1, or one of the above mutated PS1 sequences. Transfectants were identified after protein electrophoresis and Western blot analysis by means of αPS1Loop antibody (19).

Effect of Inhibitors on PS Immunoreactivity in Stably Transfected HEK293 Cells

Stably transfected cells were exposed to a series of inhibitors targeting the proteasome or other proteases. Cells were then washed, lysed in Tris buffer saline (TBS; NaCl, 140 mM; Tris, 20 mM; pH 7.5) containing 2% sodium dodecyl sulfate (TBS-SDS), then 20 μg of protein was diluted twice in the loading buffer, electrophoresed on a 12% SDS-PAGE, and Western blotted for 3 hr. Nitrocellulose sheets were capped for 45 min with skim milk (5% in TBS) and exposed over-

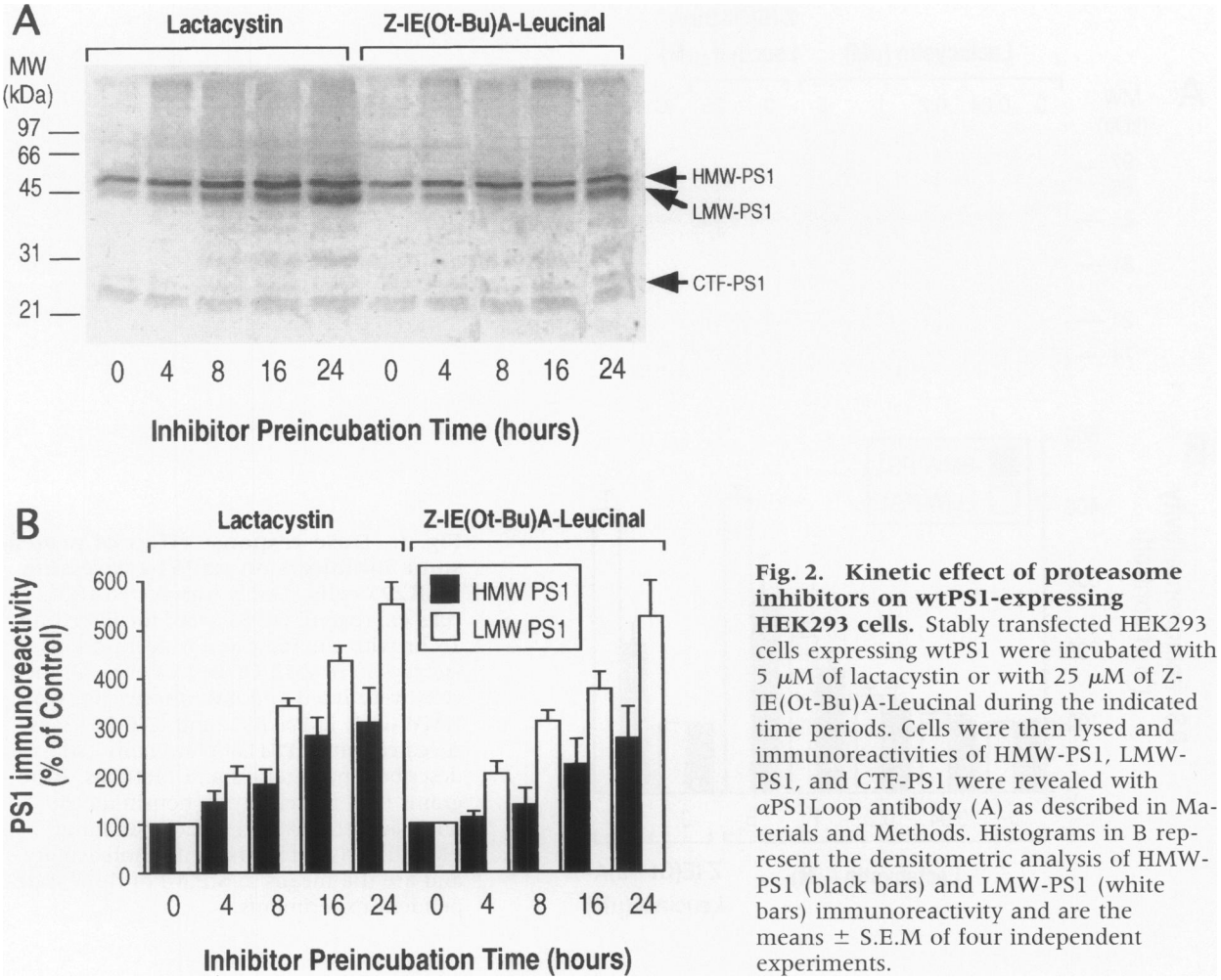

Fig. 2. Kinetic effect of proteasome inhibitors on wtPS1-expressing HEK293 cells. Stably transfected HEK293 cells expressing wtPS1 were incubated with 5 μM of lactacystin or with 25 μM of Z-IE(Ot-Bu)A-Leucinal during the indicated time periods. Cells were then lysed and immunoreactivities of HMW-PS1, LMW-PS1, and CTF-PS1 were revealed with αPS1Loop antibody (A) as described in Materials and Methods. Histograms in B represent the densitometric analysis of HMW-PS1 (black bars) and LMW-PS1 (white bars) immunoreactivity and are the means ± S.E.M of four independent experiments.

night to a 1000-fold dilution of αPS1Loop antibody or to a 5000-fold dilution of an anti-N-terminal-PS1 antibody (B14.5 provided by Bart De Strooper, Leuven, Belgium). The nitrocellulose was rinsed with TBS then incubated with adequate anti-IgGs, revealed, and quantified as previously described (15).

Immunoprecipitation of PS1 and Immunodetection of Ubiquitin

HEK293 cells overexpressing wtPS1 were grown in 35-mm dishes. Before use, cells were washed with phosphate buffer saline (PBS) and lysed in 1 ml of radioimmunoprecipitation assay (RIPA) buffer. wtPS1 was immunoprecipited with αPS1Loop antibody (1/1000 dilution) and 10 mg of protein A-Sepharose (Sigma). Immunoprecipitates were rinsed then submitted to an 8% SDS-PAGE and Western blotted onto a nitrocellulose membrane (Hybond C, Amersham) for 3 hr. Nitrocellulose sheets were capped for 45 min with skim milk (5% in TBS) and exposed overnight to a 500-fold dilution of monoclonal anti-ubiquitin antibodies (Ubi-1). The nitrocellulose was rinsed with TBS then incubated with peroxidase-coupled anti-mouse IgGs and revealed as above.

Transient Transfections, Metabolic Labeling, and Detection of Aβ40 and Aβ42

Stable transfectants overexpressing wild-type, ΔE9, or mutated PS1 were transiently transfected by calcium phosphate precipitation with 2 μg of wild-type βAPP751 cDNA. Transfection efficiency was checked by Western blot with mAb22C11 antibody (15).

After 2 days, cells were treated with proteasome inhibitors for 15 hr then metabolically

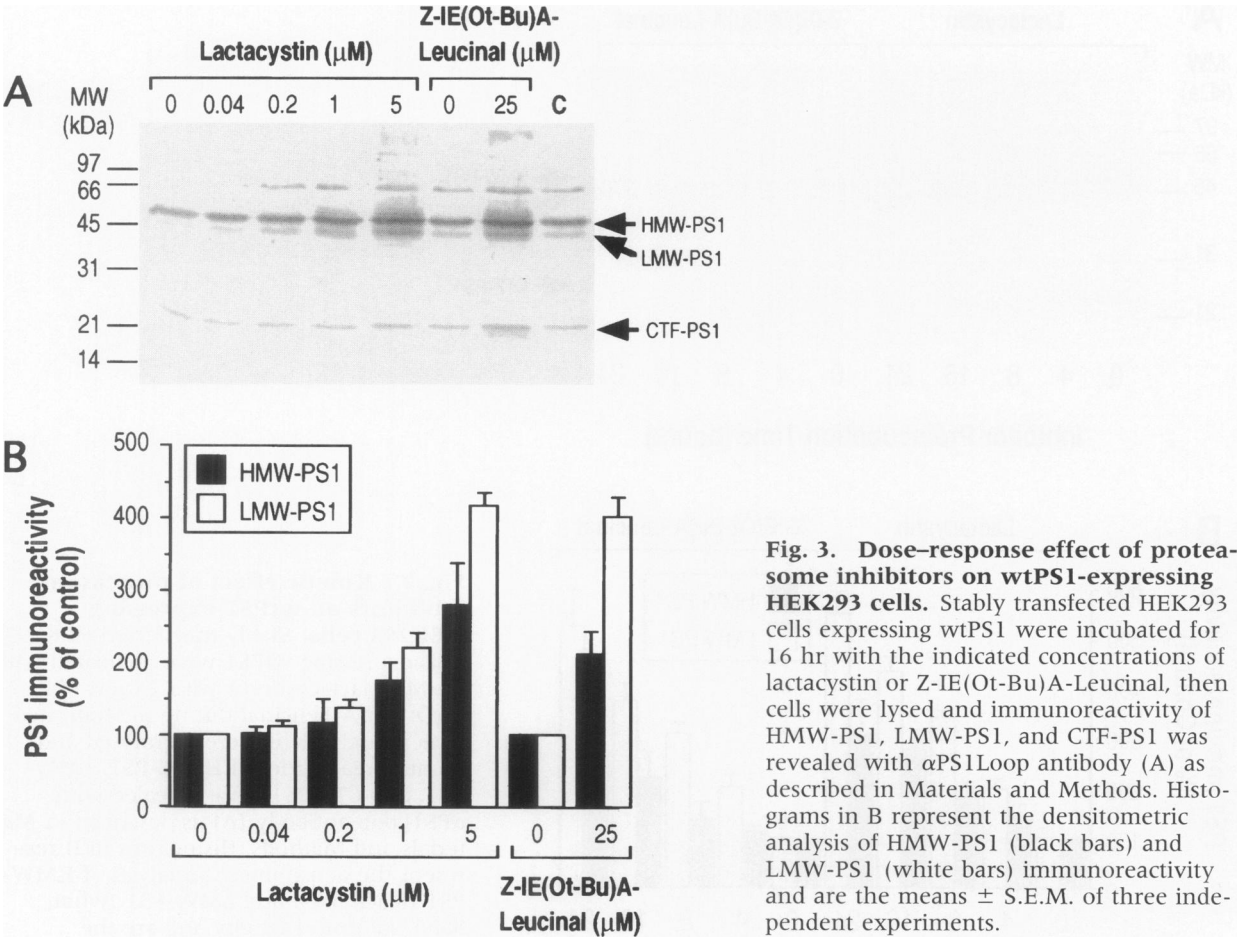

Fig. 3. Dose–response effect of proteasome inhibitors on wtPS1-expressing HEK293 cells. Stably transfected HEK293 cells expressing wtPS1 were incubated for 16 hr with the indicated concentrations of lactacystin or Z-IE(Ot-Bu)A-Leucinal, then cells were lysed and immunoreactivity of HMW-PS1, LMW-PS1, and CTF-PS1 was revealed with αPS1Loop antibody (A) as described in Materials and Methods. Histograms in B represent the densitometric analysis of HMW-PS1 (black bars) and LMW-PS1 (white bars) immunoreactivity and are the means ± S.E.M. of three independent experiments.

labeled for 6 hr as described earlier (20). Conditioned media were collected, diluted in a one-tenth volume of RIPA 10× buffer, then submitted to sequential immunoprecipitation procedures by means of FCA3542 and FCA3340 antibodies (21). Briefly, the above diluted media were incubated overnight with a 350-fold dilution of FCA3542 then further incubated for 5 hr with protein A-Sepharose. After centrifugation, the resulting supernatant was further incubated for 15 hr with a 350-fold dilution of FCA3340 and treated as above. Both pellets were resuspended with loading buffer, submitted to 16.5% Tris-tricine electrophoresis, and radioautographed as described earlier (9). Densitometric analysis was performed by phosphorImager (Fuji).

Antibodies

FCA3340 and FCA3542 specifically interact with Aβ40 and Aβ42, respectively (21). αPS1Loop recognizes the hydrophilic loop of PS1 located between its predicted sixth and seventh transmembrane domain (19). Antibody B14.5 is directed toward the N-terminus of PS1. mAb22C11 was from Boehringer, and Ubi1 was purchased from Zymed (San Francisco, CA).

Results

Western blot analysis of stably transfected HEK293 cells overexpressing wild-type and mutated PS1 indicate that αPS1Loop antibody predominantly labels a protein doublet of about 48–45 kDa, referred to as high-molecular-weight PS1 (HMW-PS1) and low-molecular-weight PS1 (LMW-PS1), respectively, as well as a product of about 25 kDa referred to as C-terminal fragment PS1 (CTF-PS1). By contrast, αPS1Loop antibody reveals a major proteic band of slightly lower molecular weight and did not label any maturated product in ΔE9-PS1-expressing cells (Fig. 1).

We previously established that a 1-μM con-

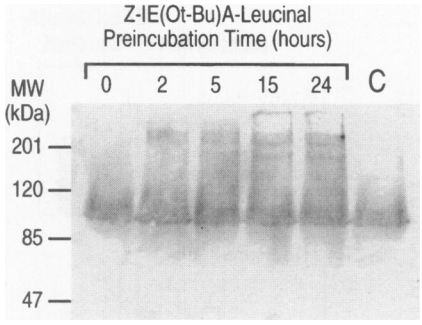

Fig. 4. Polyubiquitination of PS1 protein in HEK293 cells: effect of Z-IE(Ot-Bu)A-Leucinal. Stably transfected HEK293 cells expressing wtPS1 were preincubated with 25 μM of Z-IE(Ot-Bu)A-Leucinal during the indicated time periods, then cells were scraped, and lysed in RIPA buffer. wtPS1 was immunoprecipitated with αPS1Loop antibody (1/1000e dilution) as described in Materials and Methods. Immunoprecipitates were rinsed with RIPA buffer then electrophoresed on an 8% SDS-PAGE then Western blotted as described in Materials and Methods. Ubiquitin-like immunoreactivity was revealed with a monoclonal anti-ubiquitin antibody (Ubi-1). Control (C) corresponds to cells preincubated for 24 hr with the adequate DMSO concentration.

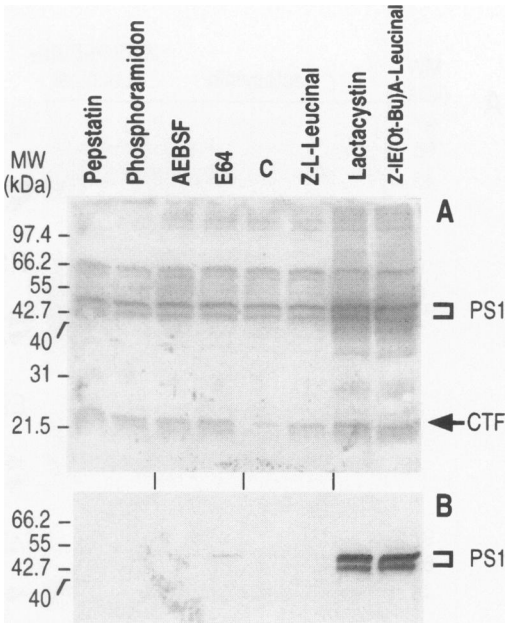

Fig. 5. Effect of various inhibitors on wtPS1-expressing HEK293 cells. Stably transfected HEK293 cells expressing wtPS1 were incubated for 16 hr without (C) or with trans-Epoxysuccinyl-L-leucylamido-(4-guanidino) butane (E64, 10^{-4} M), 4-(2-Amidoethyl)benzenesulfonyl fluoride (AEBSF, 10^{-4} M), phosphoramidon (10^{-5} M), pepstatin A (10^{-5} M), Z-L-Leucinal (2.5 10^{-5} M), Z-IE-(Ot-Bu)A-Leucinal (2.5 10^{-5} M), and lactacystin (5 10^{-6} M). Cells were lysed and immunoreactivity of PS1 holoprotein (PS1) was revealed with αPS1Loop (A) or anti-N terminal B14 (B) antibodies as described in Materials and Methods. CTF, C-terminal fragment.

centration of two proteasome inhibitors lactacystin (22) and Z-IE(Ot-Bu)A-Leucinal (23) fully abolished all the chymotryptic-like activity present in a homogenate of HEK293 cells and that all this activity could be ascribed to the proteasome as it was immunoprecipitated by specific proteasome antibodies (17). Furthermore, we recently demonstrated that a 30-min treatment of HEK293 cells with 25 μM of Z-IE(Ot-Bu)A-Leucinal fully abolished intracellular proteasome activity (16), indicating that this agent was cell permeant.

Lactacystin and Z-IE(Ot-Bu)A-Leucinal were used to examine whether they could modify wtPS1 immunoreactivity in transfected cells. Figure 2A clearly shows that both inhibitors strongly protect wtPS1 immunoreactivity, in a time-dependent manner. These agents concomittantly potentiate the recovery of both HMW-PS1 and LMW-PS1 (Fig. 2B), although the protection appears particularly strong for LMW-PS1. It should be noted that the two proteasome inhibitors also unmask a trail of PS1-related immunoreactivity that increases with time (Fig. 2A). These agents do not significantly alter the recovery of CTF-PS1 generated by these cells.

Figure 3A indicates that the treatment of wtPS1-expressing transfectants with a 25 μM concentration of Z-IE(Ot-Bu)A-Leucinal strongly enhances both HMW-PS1 and LMW-PS1 immunoreactivity. Lactacystin also elicits a dose-dependent protection of PS1 immunoreactivity (Fig. 3A), with a maximal protection at 5 μM, the extent of which resembles that elicited by 25 μM of Z-IE(Ot-Bu)A-Leucinal (Fig. 3A). Here again, both HMW-PS1 and LMW-PS1 appear concomittantly protected from the degradation process (Fig. 3B). Control experiments indicate that the addition of Z-IE(Ot-Bu)A-Leucinal during the extraction procedure did not affect PS1 immunoreactivity, therefore ruling out the possible artefactual contribution of the proteasome during this step of the procedure (Fig. 3A, lane c).

In agreement with the current knowledge on proteasome intracellular targets, we have established that immunoprecipitated wtPS1 can be labeled with anti-ubiquitin-specific antibodies (Fig. 4). Interestingly, this label is increased upon treatment of the cells with Z-IE(Ot-Bu)A-Leuci-

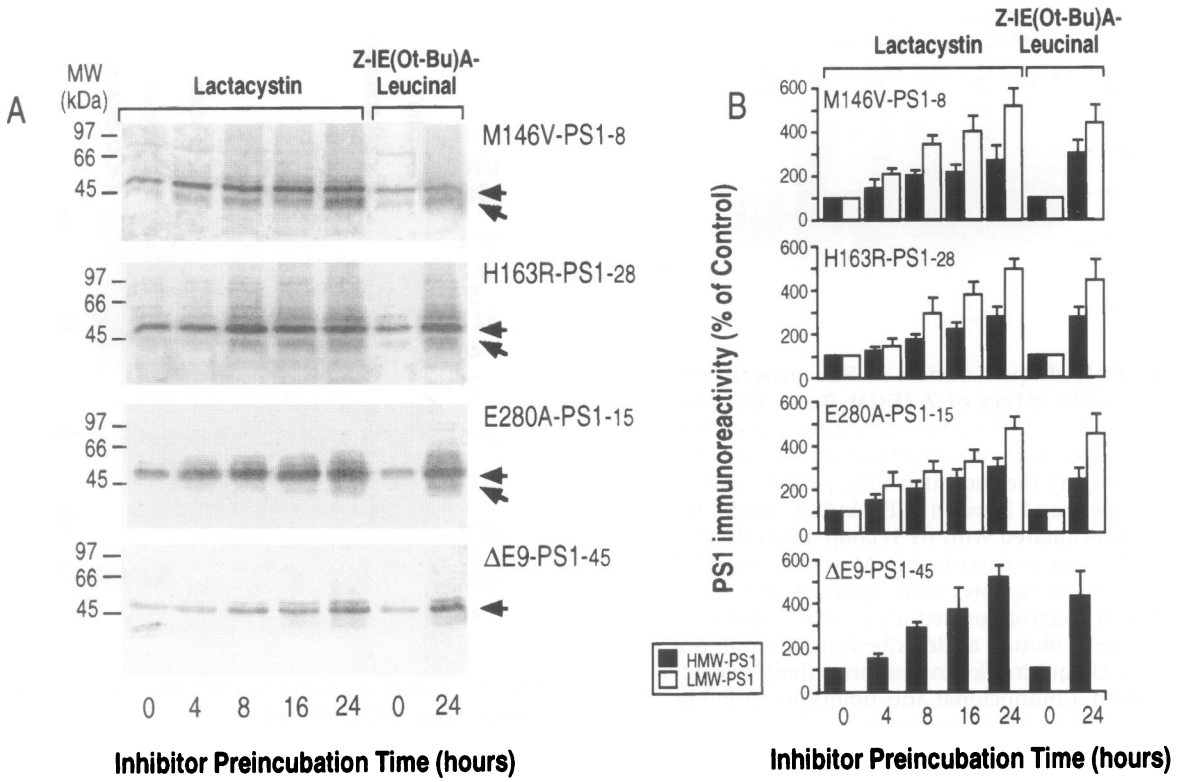

Fig. 6. Effect of proteasome inhibitors on FAD-linked PS1-expressing HEK293 cells. Stably transfected HEK293 cells expressing mutated PS1 or ΔE9-PS1 were incubated with 5 μM of lactacystin or with 25 μM of Z-IE(Ot-Bu)A-Leucinal during the indicated time periods, then cells were lysed and immunoreactivities of HMW-PS1 and LMW-PS1 (indicated by upper and lower arrows, respectively) were revealed with αPS1Loop antibody (A) as described in Materials and Methods. Histograms in B represent the densitometric analysis of HMW-PS1 (black bars) and LMW-PS1 (white bars) immunoreactivity and are the means ± S.E.M of four independent experiments.

nal (Fig. 4), confirming that polyubiquitinated wtPS1 behaves as a substrate of the proteasome in HEK293 cells.

Other inhibitors unable to affect the proteasome, such as E64, AEBSF, phosphoramidon, pepstatin, Z-L-Leucinal, or TNF-α protease inhibitor (not shown), did not modify wtPS1-like immunoreactivity (Fig. 5A) and did not affect the recovery of the CTF fragment (Fig. 5A) after visualization with αPS1Loop antibody. Interestingly, when PS1 recovery was assessed with an antibody recognizing the N-terminal region of PS1 (B14), a similar increase in PS1 holoprotein immunoreactivity was exclusively observed after cell treatment with proteasome inhibitors (Fig. 5B).

We examined the effect of the two proteasome inhibitors on the PS immunoreactivity in cells overexpressing mutated or ΔE9-PS1. Kinetic analyses indicate that lactacystin and Z-IE(Ot-Bu)A-Leucinal strongly augment PS-like immunoreactivity in all transfected cells (Fig. 6A). The extent of protection of HMW-PS1 and LMW-PS1 immunoreactivity obtained with 5 μM and 25 μM of lactacystin and Z-IE(Ot-Bu)A-Leucinal, respectively, appears very similar. Both agents led to higher protection of LMW-PS1 immunoreactivity (Fig. 6B). Independent transfectant clones expressing identical mutated PS responded similarly to the proteasome inhibitors. Table 1 documents the increased immunoreactivity of both HMW- and LMW-PS1, the latter showing particularly strong immunoreactivity.

We assessed whether the proteasome inhibitors could affect pathogenic βAPP maturation and particularly whether they could modify the ratio of Aβ42 to total Aβ (Aβ40 + Aβ42) secretion by transfected cells. Mock transfected HEK293 cells (naive cells) secrete low amounts of Aβ40 and Aβ42 (Fig. 7A, B). Z-IE(Ot-Bu)A-Leucinal increases the recovery of both Aβ spe-

Table 1. Effect of Z-IE(Ot-Bu)A-Leucinal on wild-type and FAD-linked PS1 immunoreactivity in distinct, independent transfectant clones

Cell Line	Number of Experiments	% of Control HMW-PS1	% of Control LMW-PS1
wtPS1-29	4	276 ± 69	520 ± 73
M146V-PS1-8	3	301 ± 52	442 ± 81
M146V-PS1-21	3	262 ± 55	350 ± 68
H163R-PS1-9	3	211 ± 42	399 ± 74
H163R-PS1-28	4	293 ± 37	442 ± 85
E280A-PS1-12	3	280 ± 35	425 ± 59
E280A-PS1-15	4	249 ± 45	460 ± 79
ΔE9-PS1-45	3	440 ± 110	nd

Stably transfected HEK293 cells expressing wt, mutated, or ΔE9-PS1 were incubated for 24 hr with 25 μM of Z-IE(Ot-Bu)A-Leucinal. Cells were then lysed and immunoreactivities of HMW-PS1 and LMW-PS1 were revealed with αPS1Loop antibody and quantified as described in Materials and Methods. Values are expressed as the percent of control corresponding to immunoreactivity observed in identical cells in the absence of inhibitor and are the means ± S.E.M. of the indicated number of independent experiments. nd, not detectable.

cies without affecting the Aβ42/Aβ total ratio. Overexpression of wtPS1 appears to favor Aβ40 production (Fig. 7B, D) as reflected by a lower Aβ42/Aβ total ratio when compared with naive cells (Fig. 7D). Interestingly, PS1 mutations or Exon9 deletion trigger increased secretions of both Aβ species. However, unlike for wtPS1, these FAD-linked mutations clearly favor the recovery of the more pathogenic species Aβ42. Most interesting is the fact that Z-IE(Ot-Bu)A-Leucinal further potentiates the recovery of Aβ42 (Fig. 7A) by these cells as reflected by increased Aβ42/Aβtotal ratios (Fig. 7D). Identical experiments performed with independent clones all indicate a similar favored potentiation of Aβ42 secretion by FAD-linked PS1-expressing cells upon the proteasome inhibitor (Table 2).

Discussion

We have set up stable transfectants overexpressing wild-type and mutated PS1. By means of an antibody directed toward the extracellular hydrophylic loop of PS1, we detect the overexpression of a protein doublet at 45–48 kDa (referred to as LMW-PS1 and HMW-PS1, respectively) as was previously described in several other studies (4,6,24). The same type of labeling is observed with an antibody directed towards the N-terminal part of PS1 (see Fig. 5B), confirming the identity of the detected protein as genuine PS1. It appears unlikely that these two proteic bands correspond to immature and mature PS1 species since treatment of cell lysates with endoglycosidase H does not modify the immunoreactivity pattern (not shown) in agreement with previous studies showing the absence of glycosylation of PS1 (12,25). We also observe an additional product of about 25 kDa (CTF-PS1) that likely corresponds to the C-terminal product of PS1 maturation. In line of such hypothesis, it should be noted that the overexpression of ΔE9-PS1, a nonmaturated PS1 construction (19), does not lead to the detection of such CTF-PS1 immunoreactivity (Fig. 1). The fact that the molecular weight of CTF-PS1 appears to be slightly higher than those previously reported could reflect a high phosphorylation state of this product in our conditions (26,27).

Two specific inhibitors of the proteasomal multicatalytic complex, lactacystin and Z-IE(Ot-Bu)A-Leucinal, elicit time- and dose-dependent protection of wtPS1 immunoreactivity. These agents also trigger identical protection of FAD-linked M146V-PS1, H163R-PS1, and E280A-PS1. This is not accompanied by a concommitant decrease in CTF-PS1 immunoreactivity, thereby indicating that the inhibitors more likely prevent a proteasomal contribution to PS1 degradation but not maturation. Accordingly, the same protection was observed with the cells expressing the maturation-resistant ΔE9-PS1 construction.

Interestingly, both LMW-PS1 and HMW-PS1 immunoreactivity is affected by proteasome inhibitors, although the extent of inhibition appears to be clearly higher for LMW-PS1. The nature of LMW-PS1 and HMW-PS1 is not yet clear. However, they do not correspond to distinct glycosylation states (not shown), which is in agreement with the fact that the main subcellular immunohistochemical localization of PS includes cell compartments involved early in biosynthetic pathways, such as endoplasmic reticulum and early Golgi (10–12). The current knowledge of proteasome specificity indicates that this catalytic complex triggers the degradation of intracellular polyubiquitinated proteins (28). In this context, it is interesting to note that PS1 can undergo polyubiquitination in HEK293

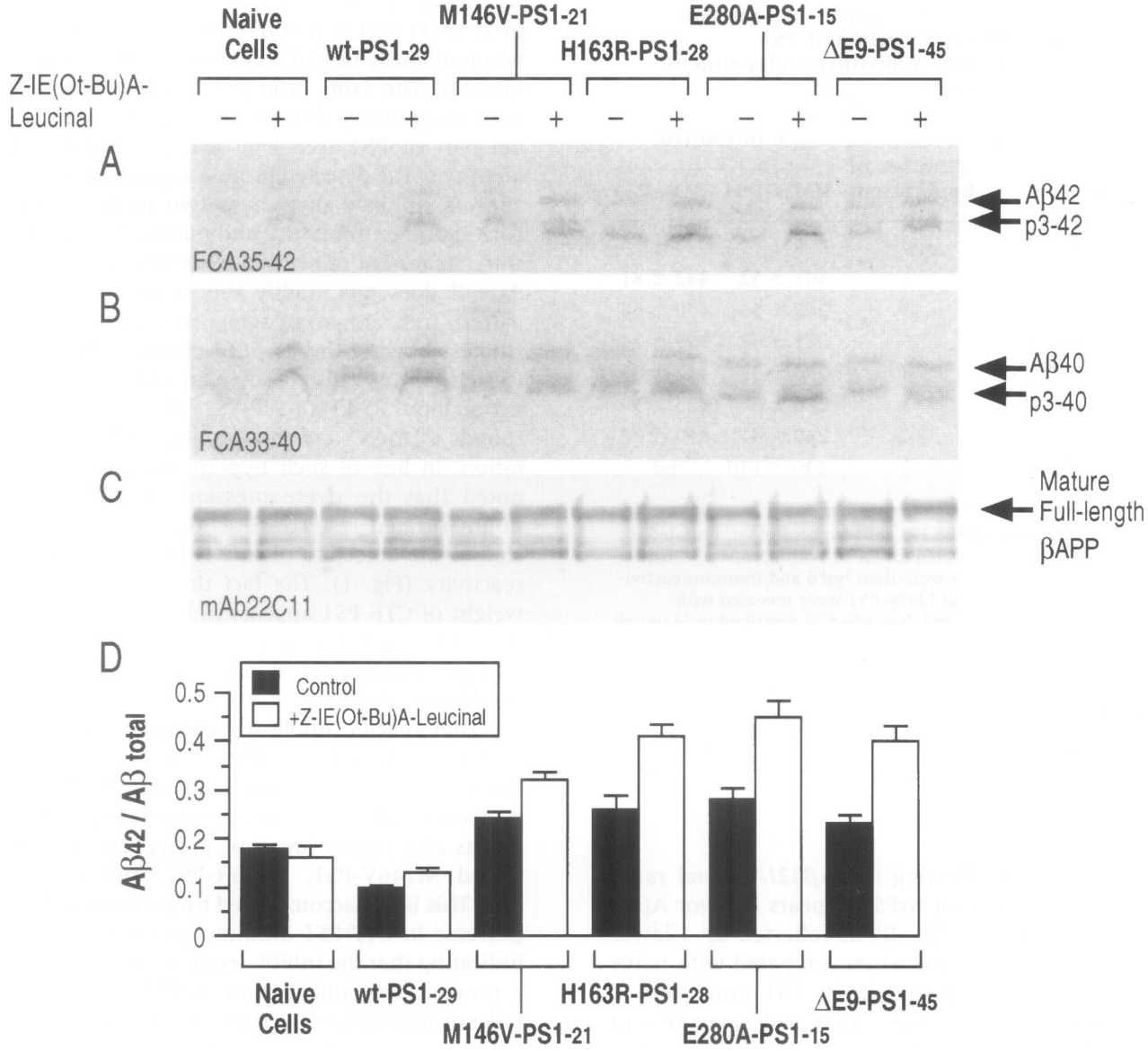

Fig. 7. Effect of Z-IE(Ot-Bu)A-Leucinal on Aβ40 and Aβ42 secretion by HEK293 cells expressing wild-type and FAD-linked PS1. Naive HEK293 cells or stable transfectants overexpressing wt or the indicated mutated PS1 proteins were transiently transfected with 2 μg of wild-type βAPP751 cDNA. Thirty-six hours after transfection, cells were incubated for 16 hr with Z-IE(Ot-Bu)A-Leucinal (25 μM), then metabolically labeled for 6 hr in the presence of the inhibitor. Conditioned media were submitted to a subsequent two-step immunoprecipitation with FCA3542 (A) and FCA3340 (B) antibodies (350-fold dilution) as described in Materials and Methods. Immunoprecipitated proteins were electrophoresed on a 16.5% Tris-tricine gel and radioautographed and analysed by densitometry. Panel D represents the ratio of Aβ42 to total Aβ (corresponding to Aβ42 + Aβ40) observed with indicated clones in the absence (control, black bars) or presence (Z-IE(Ot-Bu)A-Leucinal, white bars) of the proteasome inhibitor. Values are the means ± S.E.M. of three independent experiments. Panel C represents βAPP immunoreactivity revealed with mAb22C11 as described in Materials and Methods.

cells (Fig. 4), as was shown for PS2 (29). Several lines of evidence now suggest that although mainly cytosolic, the proteasome could also be detected in other cell compartments (30,31), including the endoplasmic reticulum and early Golgi (32), where it could contribute to the unconventional degradation of various endoplasmic reticulum-associated proteins (33).

Inhibitors targeting thiol (E64) or serine (AEBSF) proteases were unable to affect PS1 im-

Table 2. Effect of Z-IE(Ot-Bu)A-Leucinal on Aβ secretion by independent clones expressing wild-type and FAD-linked PS1

Cell Line	Aβ42/Aβ total Ratio	
	Control	+Z-IE(Ot-Bu) A-Leucinal
Naive cells	0.18 ± 0.007	0.16 ± 0.025
wtPS1-29	0.10 ± 0.001	0.13 ± 0.010 (100)
M146V-PS1-8	0.24 ± 0.017	0.32 ± 0.015 (246)
M146V-PS1-21	0.22 ± 0.020	0.27 ± 0.021 (208)
H163R-PS1-9	0.25 ± 0.007	0.34 ± 0.040 (262)
H163R-PS1-28	0.26 ± 0.030	0.41 ± 0.029 (315)
E280A-PS1-12	0.24 ± 0.030	0.46 ± 0.032 (354)
E280A-PS1-15	0.28 ± 0.024	0.45 ± 0.040 (346)
ΔE9-PS1-45	0.23 ± 0.020	0.40 ± 0.032 (308)

Naive HEK293 cells or stable transfectants overexpressing wt, mutated, or ΔE9-PS1 were transiently transfected with wtβAPP751 cDNA. Thirty-six hours after transfection, cells were incubated for 16 hr in the absence (control) or presence of Z-IE(Ot-Bu)A-Leucinal (25 μM), then metabolically labeled for 6 hr in the presence of the inhibitor. Conditioned media were submitted to a subsequent two-step immunoprecipitation procedure with FCA3542 and FCA3340 antibodies (350-fold dilution). The ratios of Aβ42/Aβ total were estimated as in Figure 7. Values are the means ± S.E.M. of three to four independent experiments. Values in parentheses represent the Aβ42/Aβ total ratio expressed as the percent of that obtained with wtPS1-expressing cells (taken as 100).

munoreactivity. Furthermore, pepstatin A also appears ineffective, indicating that the overall increase in PS1 immunoreactivity was not due to the blockade of acidic proteases located in a lysosomal compartment where they would be responsible for final catabolic processes. Finally, two more specific blocking agents were examined. Phosphoramidon, which appears to protect Aβ from intracellular degradation (34), and Z-L-Leucinal, a calpain inhibitor, were unable to modify PS1 immunoreactivity. None of the above inhibitors affect the recovery of the CTF, indicating that this product does not undergo subsequent cleavage by proteases covered by the inhibitory spectrum of these inhibitors.

PS1 mutations trigger an increased Aβ42 formation that is likely responsible for FAD-linked PS1 pathogenicity (for reviews, see refs. 35–37). Thus, we examined whether proteasome inhibitors could lead to phenotypic enhanced secretion of Aβ42 in transfected cells or could modify the ratio of Aβ42 to total Aβ (Aβ40 + Aβ42) recoveries. Our experiments show that in mock transfected cells, proteasome inhibitors lead to increased production of both Aβ40 and Aβ42 species, as indicated by identical ratios observed in control and inhibition conditions. Overexpression of wtPS1 leads to increased Aβ40 and Aβ42 recovery with a higher magnitude observed for the Aβ40 species. In this cell system, proteasome inhibitors induce a nonselective enhancement of both Aβ species, as indicated by the similar Aβ42 to total Aβ ratio. By contrast, mutations on PS1 lead to increased production of both Aβ40 and Aβ42 but clearly favor the production of the latter species as reflected by the increased Aβ42 to total Aβ ratio. Proteasome inhibitors protect mutated PS1 from degradation, thereby exacerbating Aβ42 production as reflected by an increase of this ratio. Altogether, our study suggests that the proteasome participates in the degradation of wt and mutated PS1 and directly influences Aβ production. It should be noted that direct involvement of the proteasome in Aβ degradation is unlikely, as we previously demonstrated that the purified enzyme was unable to cleave synthetic Aβ in vitro (15).

We previously established that proteasome inhibitors could increase the constitutive secretion of APPα (16), the physiological product of βAPP maturation (38). These data, together with the present study strongly suggest that the proteasome influences both α- and β/γ-secretase pathways, probably through the degradation of PS1. These catabolic events likely occur upstream to the contribution of PS1 to the maturation and/or routing of βAPP. Furthermore, the selective increased secretion of Aβ42 observed with cells expressing FAD-linked PS1 strongly supports a dysfunction in the βAPP routing brought about by these mutations. A recent study seems to indicate that the proteasome could also contribute to the degradation of wild-type presenilin 2 (29).

Whether the proteasome could serve as therapeutic target remains to be established. It is interesting to note, however, that several natural endogenous activators of the proteasome have been described (for reviews, see refs. 39,40). In the case of early-onset forms of Alzheimer's disease linked to mutations on PS1, it is thus possible to envision that selective enhancers of the proteasome activity could diminish Aβ42 formation through increased degradation of mutated PS1 and therefore slow down or arrest the neu-

rodegenerative process that is likely related to overexpression of such Aβ42 species.

Acknowledgments

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