Fatty acids increase presenilin-1 levels and γ-secretase activity in PSwt-1 cells

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Abstract   Presenilin-1 (PS1) is an important determinant of the γ-secretase activity necessary for the generation of β-amyloid (Aβ), likely the central pathogenic molecule in Alzheimer's disease. Most presenilin is rapidly degraded, and determinants of the level of the active cleaved form are unknown. We examined the influence of fatty acids on PS1 levels and γ-secretase activity using stably transfected CHO cells that express human PS1 and the human amyloid precursor protein. Cells cultured with 0.4 mM oleic acid (OA), with 0.1 mM linoleic acid, or with a triglyceride emulsion expressed increased PS1 and Aβ. This effect was independent of any secondary increase in cellular cholesterol. Cells cultured in 0.4 mM OA also exhibited significantly increased γ-secretase activity. PS1 mRNA levels were unchanged, and pulse-chase experiments indicated that OA slowed presenilin holoprotein degradation. Nontransfected human neuroblastoma cells also showed increased presenilin when cultured in 0.4 mM OA.▮▮ Lipids may be important biological determinants of PS1 level and γ-secretase activity.—Liu, Y., L. Yang, K. Conde-Knape, D. Beher, M. S. Shearman, and N. S. Shachter. **Fatty acids increase presenilin-1 levels and γ-secretase activity in PSwt-1 cells.** *J. Lipid Res.* 2004. **45:** 2368–2376.

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Alzheimer's disease (AD) possesses a prominent heritable component of risk. In the case of early-onset AD, this commonly represents mutation of the presenilin-1 (PS1) gene. Mutations of presenilin-2 and of the amyloid precursor protein (APP) have also been observed (1). In the case of late-onset AD, the most important predictors are the ε2/ε3/ε4 alleles of apolipoprotein E (apoE) (2). The isoforms of apoE possess a long list of functional differences whose relative importance in contributing to AD risk has been difficult to weigh (3). However, the accumulation of β-amyloid (Aβ), a 40–42 amino acid peptide cleavage product of APP, has been proposed to represent the central pathogenic abnormality in AD, and all hereditary factors affecting AD risk are expected to influence this pathway (4).

Aβ is a minor product of APP processing that is released after cleavage by so-called β-secretase and γ-secretase. A third APP-cleaving enzyme, so-called α-secretase, cleaves within the Aβ sequence and thereby decreases Aβ formation. Increased neuronal cholesterol has been associated with decreased activity of the principal α-secretase and with increased Aβ (5–7). The molecular identity of β-secretase has been established, and the influence of its level of activity on Aβ generation is an area of ongoing investigation (8, 9). Evidence has accumulated that PS1, a polytopic membrane protein, is a prerequisite for the major part of γ-secretase activity (10, 11). Presenilin mutations appear to increase γ-secretase cleavage of APP and to increase production of the highly amyloidogenic Aβ42 (12). Interestingly, overexpression of mutant PS1 in neuronal cells produced a prominent decrease in the number and length of neurites, which may represent a cellular phenotypic marker of the activity of the presenilin pathway (13, 14).

Most full-length presenilin is degraded in the proteasome soon after synthesis (15, 16). A small fraction acquires biological activity via transfer from the endoplasmic reticulum (ER) to the Golgi and incorporation into a high-molecular-weight complex, normally in association with cleavage and self-association to a heterodimer (17, 18). Heterodimer presenilin present in high-molecular-weight complexes has a greater than 10-fold longer half-life (~24 h) than the holoprotein (19). The rates of degradation and cleavage of presenilins are regulated by the level of an unknown cellular factor (20).

Abbreviations: Aβ, β-amyloid; AD, Alzheimer's disease; apoE, apolipoprotein E; APP, amyloid precursor protein; CD, cyclodextrin; CMV, cytomegalovirus; ECL, enhanced chemiluminescence; GST, glutathione S-transferase; LA, linoleic acid; MTT, methylthiazoltetrazolium; OA, oleic acid; PS1, presenilin-1; PS1-CTF, C-terminal fragment of cleaved PS1; PS1-NTF, N-terminal fragment of cleaved PS1; SREBP, sterol regulatory element binding protein; TG, triglyceride.

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A series of investigations from Pitas and coworkers (21) noted that overexpression of apoE4 produced a marked decrease in the number, extension, and branching of neurites. In contrast, overexpression of apoE3 led to an apparent increase in the number and extension of neurites, but with a modest decrease in branching. These phenotypic alterations were dependent on the presence of an exogenous source of lipid, in particular on a source of lipid containing triglycerides (TGs), and were abolished by interventions that blocked lipoprotein uptake (22). Given the similarities between the mutant presenilin-overexpression and the apoE4-overexpression cellular phenotypes and the dependence of the apoE-related phenotypes on the presence of a source of TGs, we hypothesized that the delivery of TGs or fatty acids was important as a determinant of presenilin processing to the active heterodimeric form. This hypothesis was recently strengthened by the observations of Peng et al. (23) that apoE-null mouse astrocytes transfected with apoE4 secrete lipoproteins that are enriched in TGs compared with astrocytes expressing apoE3 and that the apoE4 and apoE3 lipoproteins exhibit differences similar to those previously described in their stimulation of neurite outgrowth.

MATERIALS AND METHODS

Cell culture

PSwt-1 CHO cells were a gift of Dennis J. Selkoe (24). These cells are stably transfected with wild-type human PS1 and wild-type human APP full-length cDNAs, both under the regulation of the cytomegalovirus (CMV) immediate-early gene promoter. To maintain both transgenes, cells were cultured in MEM (Gibco) supplemented with 10% FBS (Gibco), 200 µg/ml G418 (Gibco), and 2.5 µg/ml puromycin (Calbiochem). SK-N-DZ human neuroblastoma cells (catalog number CRL-2149) were obtained from the ATCC and were cultured according to the instructions of the supplier. All cells were cultured on six-well plates, with concentrations as indicated of supplemental fatty acids, soybean oil emulsion (Intralipid; NDC 0338-0491-03; Fresenius Kabi, Clayton, NC), cyclodextrin (CD)-solubilized cholesterol (C3045; Sigma),
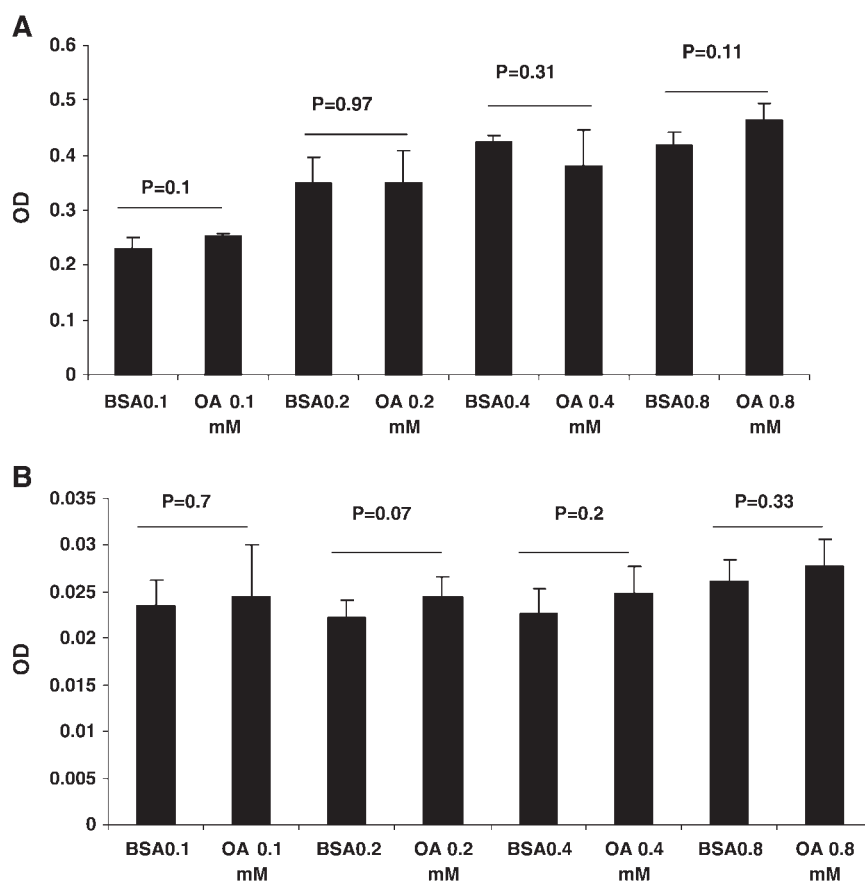
Fig. 1. Effect of oleic acid (OA) on PSwt-1 cell proliferation and viability (48 h of culturing in OA, compared with BSA control). A: Methylthiazoltetrazolium (MTT) assay. No difference in MTT assay was observed in cells cultured in up to 0.8 mM OA, compared with controls administered the corresponding amount of BSA without OA (48 h of culturing in OA, compared with BSA control). Three separate plates (N = 3) were analyzed for each condition. B: Neutral Red uptake assay. Neutral Red retention was positively correlated with cell viability. No significant difference in Neutral Red retention was observed in cells cultured in up to 0.8 mM OA, compared with controls administered the corresponding amount of BSA without OA. A trend toward greater Neutral Red retention in OA-treated cells did not reach statistical significance (48 h of culturing in OA, compared with BSA control). Six separate plates (N = 6) were analyzed for each condition. OD, optical density.

or hydroxypropyl-β-CD (33259-3; Aldrich Chemical Co., Milwaukee, WI). We performed experiments with 0.5% hydroxypropyl-β-CD used as a cholesterol acceptor (25) to increase cellular TG using 0.4 mM oleic acid (OA) while minimizing any effect of OA to increase cellular cholesterol. The fatty acid composition of Intralipid is 50% linoleic, 26% oleic, 10% palmitic, 9% linolenic, and 3.5% stearic. Media were renewed every 24 h. All test media supplements (fatty acids, etc.) were present for 48 h before cell harvesting. When supplementing cells with fatty acids, control cells always received an equivalent amount of supplemental BSA.

Fatty acid preparations

All fatty acids [OA, O-1008; linoleic acid (LA), L-1012; and stearic acid, S-4751] were from Sigma. A 16 mM fatty acid-BSA complex (5:1) stock was prepared for each fatty acid, as described (26). OA preparations were stored at $-70°C$ and were used for up to 6 months. LA and stearic acid preparations were always prepared and used fresh. Fatty acid stocks were tested for oxidation using the PeroxiDetect Kit (PD-1; Sigma). This kit enables quantitation of aqueous and organic hydroperoxides (27). There was no evidence of lipid peroxidation above control levels in new preparations of stearic acid, OA, or LA or in OA preparations that had been stored for 9 months. A modest increase of lipid hydroperoxides above baseline was noted in one (of two) OA preparations that been stored for 3 years (data not shown).

Cells grew equally well (cell protein concentration) and maintained normal morphology at all concentrations of fatty acids used in the experiments, except as described. Cell viability was determined both by the methylthiazoltetrazolium (MTT) dye assay method (28), performed using the Cell Growth Determination Kit (M0283 and M0408; Sigma), and by the Neutral Red uptake method (29), performed using the In Vitro Toxicology Assay Kit, Neutral Red Based (TOX-4; Sigma). Three separate plates (N = 3) were analyzed for each condition for the MTT assay, and six separate plates (N = 6) were analyzed for each condition for the Neutral Red uptake assay.

Lipid assays

Assays of cellular lipids were performed as described (30). All lipid data are reported normalized by cell protein (μg/mg cell protein).
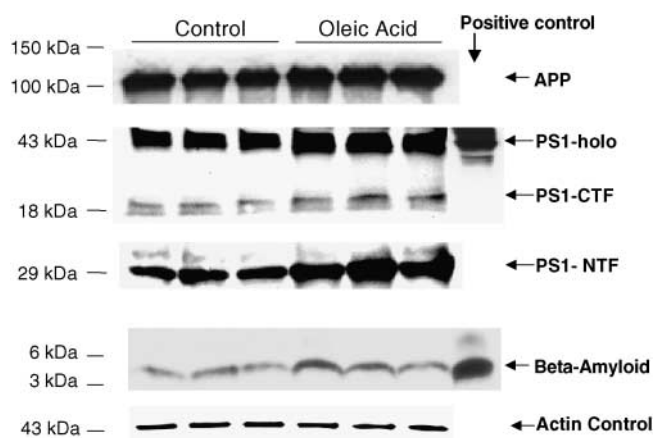

Fig. 2. Immunoblot analysis. Amyloid precursor protein (APP), presenilin-1 (PS1), and β-amyloid (Aβ) in PSwt-1 cells exposed to 0.4 mM OA [48 h of culturing in OA (N = 3), compared with BSA control (N = 3)]. Immunoblot to actin was performed as a loading control. CTF, C-terminal fragment; holo, holoprotein; NTF, N-terminal fragment. Fifty micrograms (protein) of cell lysate was loaded.

Preparation of cell extracts

Cells were lysed and cell extracts were prepared as described (31).

Immunoblots

A polyclonal goat anti-human PS1 primary antibody detected full-length, N-terminal fragment and C-terminal fragment PS1 (RDI-PRESEN1abG; Research Diagnostics, Inc., Flanders, NJ). The N-terminal fragment of human PS1 was also detected using a rat monoclonal antibody (PS12-M; Alpha Diagnostic International, San Antonio, TX). Purified glutathione S-transferase (GST)-PS1 protein used as a positive control was a gift from Nikolaos Tezapsidis (32). APP and Aβ were detected on 50 μg of cell protein from whole-cell lysates via SDS-PAGE/immunoblot. Goat anti-N-terminal human APP (RDI-AMYRREabG; Research Diagnostics, Inc.) was used for APP. A mouse monoclonal antibody that recognized human Aβ 1-40/42 (MBL M046-3; Medical and Biological Laboratories Co., Ltd., Watertown, MA) was used for the Aβ immunoblot. A monoclonal antibody that detected a broad range of actin isoforms (Mouse-anti-actin, sc-8432; Santa Cruz Biotechnology, Santa Cruz, CA) was used as a loading control.

All assays were done at least in triplicate (specified in each case). Immunoblot membranes were analyzed by fluorography using enhanced chemiluminescence (ECL; RPN2106; Amersham Pharmacia Biotech UK, Ltd.), exposure to film, and quantitated (optical density) using a digital scanner. Different gels were exposed for different amounts of time, and different autoradiograms were scanned at different resolutions, leading to different orders of arbitrary units. No absolute quantitations are reported. Each figure shows only relative amounts between the control and experimental conditions.

PS1 pulse-chase and immunoprecipitation

Presenilin pulse-chase studies were performed as described (24). PS1 was immunoprecipitated according to a described method (33) using the polyclonal goat anti-human PS1 antibody (RDI-PRESEN1abG; Research Diagnostics, Inc.). Constant amounts of TCA-precipitable counts were used to control for the extent of labeling of the entire protein pool. Human actin was immunoprecipitated from the same samples as a control. PS1 immunoprecipitation was also performed in the presence of purified GST-PS1 protein as a control for nonspecific effects of the PS1 antibody.

RNase protection assay

RNase protection assay for human PS1 was performed on pooled total RNA from six plates of cultured cells as described previously (34). The human PS1 cDNA was obtained from Research Genetics (clone 51014). A 180 bp fragment extending from nucleotides 1 to 180 was obtained by PCR of this clone using the following upstream and downstream primers, respectively: 5′-CAG-CAAGCTTATGATGGCGGGGTTCAGTGAG-3′ and 5′-CTGGAAT-
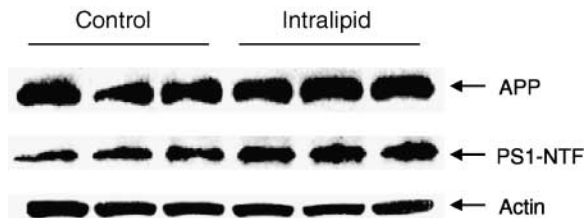

Fig. 3. Effect of Intralipid (50 μg/ml) on APP, PS1-NTF, and actin in PSwt-1 cells [48 h of culturing in Intralipid (N = 3), compared with control (N = 3)].

PCR products were digested with *Hin*dIII and *Eco*RI and subcloned into pGEM9. The pGEM9-PS1 DNA construction was linearized with *Hin*dIII and transcribed with SP6 to produce a 200 bp probe. Simultaneous hybridization was performed for PS1 and the 18S rRNA subunit, which was used to normalize the PS1 signal.

γ-Secretase in vitro membrane assay

In vitro γ-secretase activity assay was performed as described (35), with modification (36). The assay quantitates γ-secretase as generated Aβ and is reported in arbitrary units from the IGEN ECL autoanalyzer used to quantify Aβ.

Statistics

All values are presented as means ± SD. All statistical comparisons were by two-tailed *t*-test.

RESULTS

OA increased cellular presenilin and Aβ in transfected cells

Experiments were performed using a well-described cell culture model: CHO cells that had been stably transfected to overexpress both wild-type human PS1 and wild-type human APP. Cells were cultured in control medium and media supplemented with 0.1, 0.2, or 0.4 mM OA (N = 3 for each condition). Increasing concentrations of OA led to a progressive increase in cellular TGs, as follows: control, 10.5 ± 1.8 (μg/mg cell protein); 0.1 mM OA, 17.9 ± 2.5; 0.2 mM OA, 39.4 ± 2.6; 0.4 mM OA, 56.9 ± 11.4 ($P <$ 0.01 vs. control for each). In contrast, cellular cholesterol (μg/mg cell protein) only increased at the 0.4 mM OA level (65.4 ± 10.9 vs. 40.2 ± 7.3 in controls; $P <$ 0.01).

The MTT assay (**Fig. 1A**) and the Neutral Red uptake assay (Fig. 1B) were performed to determine cell proliferation and viability in these cells. No differences were observed between cells cultured in supplemental OA and cells supplemented with the corresponding amount of BSA.

Cellular levels of APP, of Aβ, and of PS1 along with its cleavage products were determined by immunoblot analysis in cells exposed to 0.4 mM OA (**Fig. 2**). Positive controls for PS1 and Aβ are included to document antibody reactivity. There was a prominent increase (optical density) in full-length PS1 [3,297 ± 29 (OA) vs. 2,219 ± 142 (control); $P <$ 0.01], in the N-terminal fragment of cleaved PS1 [PS1-NTF; 3,022 ± 462 (OA) vs. 2,023 ± 339 (control); $P <$ 0.04], and in the C-terminal fragment of cleaved PS1 [PS1-CTF; 1,874 ± 76 (OA) vs. 1,328 ± 119 (control); $P <$ 0.04]. Aβ levels also increased [1,350 ± 191 (OA) vs. 933 ± 179 (control); $P <$ 0.01]. However, APP also increased [2,722 ± 356 (OA) vs. 1,917 ± 248 (control); $P <$ 0.001], indicating the likely contribution of effects on APP synthesis or processing to the increase in Aβ. Of note, the cellular content of a control protein, actin, was not significantly altered by OA treatment, and the statistical conclusions were not changed by adjustment for actin. Lower concentrations (0.1 and 0.2 mM) of OA did not affect APP or Aβ levels and did not reproducibly affect PS1 levels (data not shown). Because cellular cholesterol increased with 0.4 mM OA but not with lower concentrations, we performed a series of experiments to determine which of these two lipid species could be driving the observed increase in PS1. Because full-length PS1, PS1-CTF, and PS1-NTF always changed in tandem, subsequent experiments only tracked PS1-NTF.

Isolated cellular enrichment with TG increased presenilin and Aβ but isolated cholesterol enrichment did not

We used cholesterol complexed to CD to deliver cholesterol to the cells under conditions that produced an increase in cellular cholesterol without increasing cellular TG. In pilot experiments with varying concentrations, we found that 4 μg/ml cholesterol produced increased cellular cholesterol with a very modest increase in TG, whereas higher concentrations increased cellular TG consistently. An isolated increase in cellular cholesterol produced by 4 μg/ml cholesterol in the medium (to 77.8 ± 11.0 vs. 51.1 ± 2.5 in controls, as μg cholesterol/mg cell protein; $P <$ 0.001) led to no statistically significant increase in PS1 [2,988 ± 213 (cholesterol-treated) vs. 2,489 ± 259 (control); P = NS] or APP [1,824 ± 6 (cholesterol-treated) vs. 1,745 ± 99 (control); P = NS]. There was no increase in
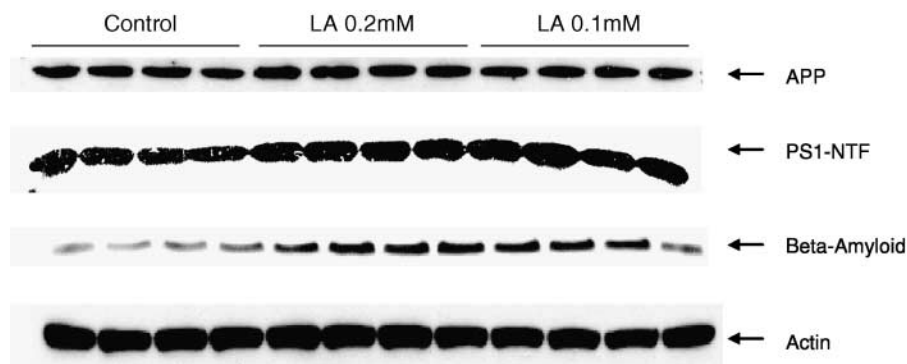

Fig. 4. Immunoblot showing effects of 0.1 mM (N = 4) and 0.2 mM (N = 4) linoleic acid (LA) on APP, PS1, and Aβ in PSwt-1 cells [48 h of culturing in LA, compared with BSA control (N = 4)]. Immunoblot to actin was performed as a loading control. Fifty micrograms (protein) of cell lysate was loaded.

Aβ [677 ± 70 (cholesterol-treated) vs. 745 ± 52 (control); P = NS; N = 3 for each condition].

We also performed experiments with a high concentration (0.5%) of CD, used as a cholesterol acceptor, to increase cellular TG using 0.4 mM OA [46.2 ± 6.6 (OA/CD TG) vs. 9.7 ± 0.32 (control TG); $P < 0.0001$] while minimizing any effect of OA to increase cellular cholesterol [39.8 ± 2.1 (OA/CD cholesterol) vs. 34.2 ± 1.8 (control cholesterol); $P < 0.02$; N = 3 for each condition]. The relatively isolated increase in cellular TG attributable to OA/CD treatment led to increased PS1 [3,069 ± 21 (OA/CD) vs. 2,489 ± 259 (control); $P < 0.02$], APP [2,009 ± 26 (OA/CD) vs. 1,745 ± 99 (control); $P = 0.01$], and Aβ [861 ± 38 (OA/CD) vs. 745 ± 52 (control); $P < 0.04$].

We attempted to replicate the effects of OA without administering fatty acid directly by using a commercially available TG emulsion (Intralipid) that is devoid of cholesterol. Intralipid (50 μg/ml, as TG) increased cellular TG to a similar extent as 0.4 mM OA and did not increase cholesterol at all (data not shown). Intralipid (**Fig. 3**) also led to increased PS1 [726 ± 27 (control) vs. 810 ± 42; $P < 0.04$] and APP [610 ± 57 (control) vs. 762 ± 53; $P = 0.028$]. Actin was similar [669 ± 37 (control) vs. 673 ± 15; P = NS].

LA is more potent than OA in increasing cellular presenilin despite an identical effect on cellular lipid stores

Because of the difference in fatty acid composition between Intralipid and OA, we sought to determine whether the specific fatty acid used influenced the potency of the effect on PS1 levels. Concentrations of stearic acid (18:0) up to 0.1 mM had a lesser effect on increasing cellular TG and had no effect on PS1 (data not shown). Higher concentrations could not be used because of increased cell death, as has been described (37). LA (18:2) produced no
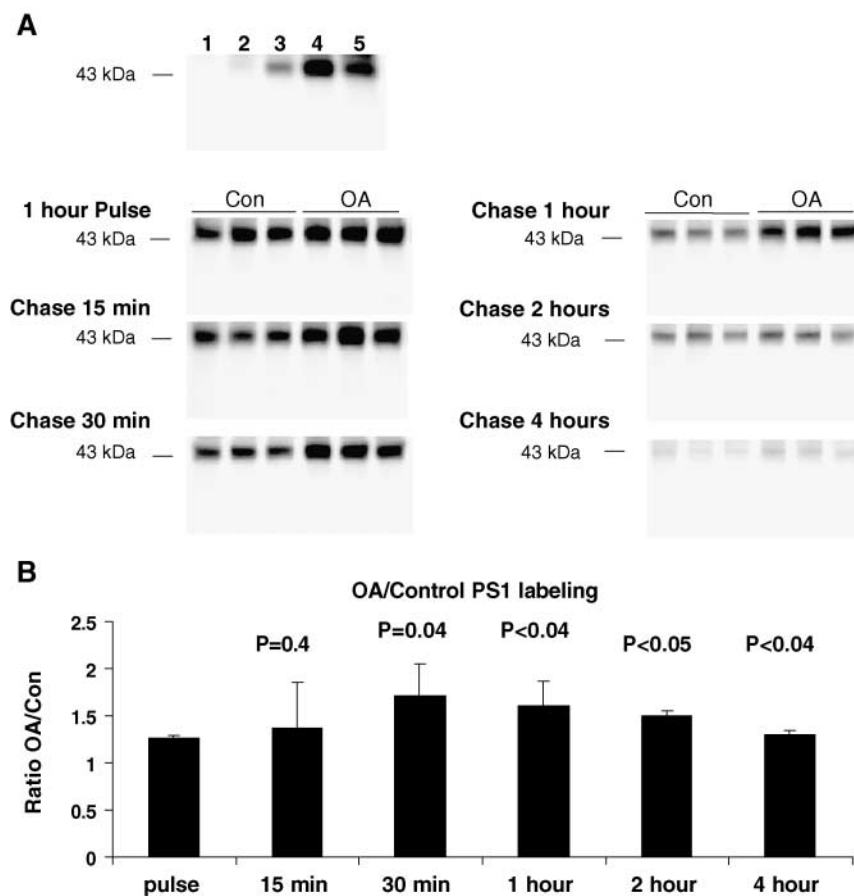

Fig. 5. A: PS1 pulse-chase in the presence or absence of 0.4 mM OA in PSwt-1 cells [48 h of culturing in OA, compared with BSA control (Con)]. A series of immunoprecipitation controls is shown at the top. All of these samples reflect constant amounts of TCA-precipitable counts. Lane 1, lysate only, no first antibody; lane 2, actin antibody; lane 3, glutathione *S*-transferase (GST)-PS1 control (mouse anti-human PS1 antibody preabsorbed with recombinant GST-PS1 when preparing the antibody bead); lane 4, mouse anti-human PS1 antibody; lane 5, goat anti-human PS1 antibody. Immunoprecipitated labeled PS-1 after pulse and chase periods as indicated are shown in the lower part of the figure. Constant amounts of TCA-precipitable counts were used for immunoprecipitation of PS1 (N = 3 for controls and N = 3 for OA-treated cells). B: The ratio of immunoprecipitated labeled PS-1 in OA-treated cells (N = 3) vs. control cells (N = 3) is shown for the various time points. Changes in this ratio over the course of the chase period reflect differences in the rate of degradation of presenilin in OA-treated vs. control cells.

evident toxicity, and the effect of 0.1 mM OA and 0.1 mM LA on cellular lipid stores was the same (data not shown).

Immunoblots (**Fig. 4**) showed a clear effect of both 0.2 mM and 0.1 mM LA to increase PS1: 0.2 mM LA yielded an optical density of 971 ± 53 vs. 867 ± 18 in controls ($P < 0.01$); 0.1 mM LA yielded 979 ± 70 ($P = 0.02$ vs. controls). APP also increased in 0.2 mM LA [753 ± 39 vs. 648 ± 57 (control); $P = 0.02$] but not in 0.1 mM LA (683 ± 18; $P =$ NS vs. control). Interestingly, Aβ increased in both 0.2 mM LA [356 ± 27 vs. 269 ± 17 (control); $P = 0.001$] and 0.1 mM LA (328 ± 21; $P = 0.01$ vs. control). There was no consistent effect at lower concentrations (0.05 mM LA; data not shown).

Increased cellular presenilin is associated with decreased degradation of full-length PS1

The kinetics of PS1 production were determined in pulse-chase experiments (**Fig. 5**). After culturing in the presence or absence of 0.4 mM OA, the cells were metabolically labeled with [^{35}S]methionine for 1 h and then chased with unlabeled media containing the same concentration of fatty acid. Radiolabeled PS1 was immunoprecipitated from cell extract at specified time points. Constant amounts of TCA-precipitable counts were used for immunoprecipitation to control for the rate of [^{35}S]methionine incorporation into other proteins. Under these conditions, only the full-length, rather than the cleaved, PS1 is labeled to sufficient specific activity that it can be readily visualized, since the full-length form turns over much more rapidly and only a small fraction of full-length PS1 progresses to the cleaved form. There was increased labeling of full-length presenilin during the 1 h pulse in OA-treated cells (background-subtracted optical density: 121 ± 7 in controls vs. 150 ± 12 in OA-treated cells). However, there was an increase in the relative amount of PS1 in the OA-treated cells at successive time points. These data indicate that the rate of degradation of newly synthesized full-length PS1 appears

to be decreased. This would allow more full-length PS1 to progress onto the cleaved active form that contributes to the generation of Aβ.

RNase protection assay of PS1 mRNA was performed, with an 18S rRNA control, and revealed no difference in PS1 mRNA levels in OA-treated versus untreated cells, supporting the apparent posttranscriptional mechanism and arguing against any important effect of OA on the CMV promoter used to regulate the expression of the PS1 transgene (**Fig. 6**).

Native presenilin in nontransfected neuronal cells is also increased by OA

In addition, native PS1 was assayed in the SK-N-DZ human neuroblastoma cell line. These cells also showed increased levels of cleaved PS1 with OA treatment [$1,154 \pm 40$ (OA) vs. 618 ± 121 (control); $P < 0.001$], supporting our findings in transfected cells (**Fig. 7**).

OA leads to increased γ-secretase activity in transfected cells

Cells were cultured in control medium and in medium supplemented with 0.4 mM OA. The γ-secretase activity in whole cell membranes was $5,839 \pm 710$ in control cells vs. $8,275 \pm 492$ in OA-treated cells ($P < 0.0001$).

DISCUSSION

The level of cleaved presenilin is highly regulated and, along with α-secretase activity and β-secretase activity, is a major determinant of Aβ formation (6, 38). Although α-secretase activity is known to be regulated by cholesterol and other factors, the regulatory determinants of presenilin processing are unknown (7, 20). Based on our current findings, we believe that these determinants are very likely to include lipids. In stably transformed CHO cells expressing human PS1 and APP, increased fatty acid delivery increased the levels of both uncleaved and cleaved PS1 and biochemical γ-secretase activity. No homeostatic rationale for the regulation by lipid of the level of γ-secretase is known. However, presenilin mediates the intramembranous proteolytic processing of a variety of proteins besides APP, including the low density lipoprotein-related protein (39) and the apoE receptor-2 (40). These receptors, among other functions, have prominent roles in neuronal lipoprotein uptake (41).
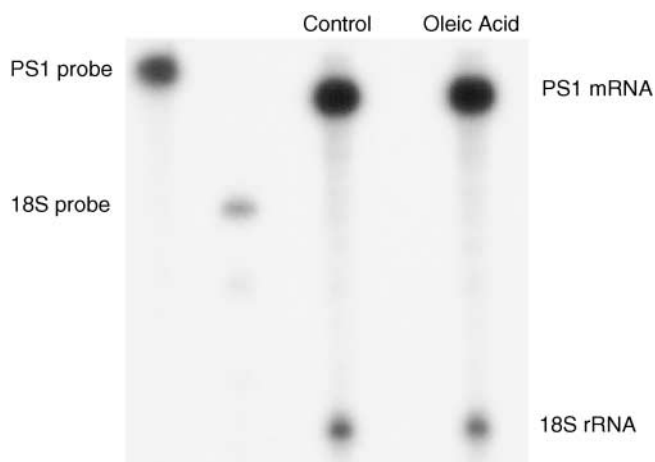

Fig. 6. RNase protection assay of PS1 mRNA in control PSwt-1 cells and in cells treated with 0.4 mM OA. Simultaneous hybridization with an 18S rRNA probe was performed as a control. Pooled RNA from six plates for each sample was used.
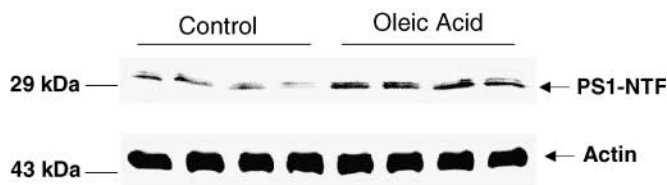

Fig. 7. Immunoblot for PS1 in control SK-N-DZ human neuroblastoma cells (N = 4) and in cells treated with 0.4 mM OA (N = 4; 48 h of culturing in OA, compared with BSA controls). Immunoblot for actin was performed as a control.

A significant increase in PS1 level was demonstrated for 0.4 mM OA, 0.4 mM OA/CD, 0.1 mM LA and Intralipid. Of these, 0.1 mM LA and Intralipid had no effect on cellular cholesterol and 0.4 mM OA/CD had very little effect. It appears clear that the increases in PS1 that we observed were the result of fatty acids, TG, or distal metabolites and cannot be accounted for by concurrent changes in cholesterol. In support of the biochemical significance of these observations, supplementation with 0.4 mM OA produced a large increase in enzymatic γ-secretase activity. In addition, increased PS1 was also observed in nontransfected neuronal cells that were supplemented with 0.4 mM OA.

Although the increase in PS1 appeared entirely attributable to fatty acids, TG, or a distal metabolite(s), the increase in Aβ that we also observed may not have been entirely attributable to increased PS1. We observed a modest increase in cellular APP with TG enrichment, similar to the increase that others have observed in the context of profound cholesterol enrichment and that has been attributed to a decrease in α-secretase activity (5). This may have played a role in increasing Aβ. However, increased Aβ was also observed with lower doses of LA that did not affect the level of APP.

It should be noted that butyrate, a fatty acid, is known to activate the CMV immediate early promoter (42). Therefore, it is possible that increased fatty acids could have produced a regulatory influence on the heterologous CMV promoter used to regulate the expression of both the APP and PS1 transgenes. However, the observation of unchanged mRNA levels in the OA-treated cells, the pulse-chase studies supporting a posttranslational mechanism for increased PS1, and, above all, the observation of increased native PS1 in a nontransfected neuronal cell line make such a supposition exceedingly unlikely.

The precise biochemical mechanisms of regulated degradation of full-length PS1, of the specific decrease in full-length PS1 degradation that we observed, and of the consequent increase in the generation of cleaved PS1 (and in γ-secretase activity) remain unclear but may occur via effects on folding (43). Regulation by lipid availability of the folding and posttranslational degradation of an ER-translocated protein is a well-established process that has been extensively studied in the case of apoB (44). Lipids may also directly promote presenilin maturation. In this regard, it has been proposed that transfer to the high-molecular-weight form, and not cleavage itself, is the regulated aspect of presenilin activation (45). The steps in the processing of the sterol regulatory element binding proteins (SREBPs) constitute the best-characterized pathway of lipid-regulated proteolysis (46). Presenilin activation bears similarities to the activation of SREBP, in which the regulated step appears to be SREBP cleavage-activating protein (SCAP)-mediated budding from the ER with transfer to a site in the Golgi where SREBP is cleaved (47, 48). SREBP activation is influenced more potently by polyunsaturated fatty acids than by monounsaturated fatty acids (49, 50), as we observed in the case of PS1, suggesting that the similarity may extend to the lipid-sensing mechanisms regulating the activation of these proteins. Enzymatic γ-secretase activity may also be directly influenced by cholesterol, al-

though this remains unclear (51, 52). Abnormal cholesterol trafficking has been shown to influence γ-secretase activity but not presenilin levels (48, 53).

The effects of fatty acids or TG on presenilin biology have not been previously reported. However, the prior reports of a harmful influence of dietary fat on AD risk may indicate possible clinical significance for our observations (54, 55). Our results may also be relevant to understanding the risk conferred by the apoE ε4 allele. It is the lipid transport properties of apoE, and the allelic differences in that regard, that have been most clearly defined (56). The apoE4 allelic isoform has been documented to have higher affinity for large TG-rich lipoproteins (57, 58). We have observed that cultured human astrocytes do secrete such particles (Y. Liu, L. Yang, T. M. Forte, J. W. Chisholm, J. S. Parks, and N. S. Shachter, unpublished data). The increased delivery of cholesterol and TGs from lipoproteins to neurons, along with a subsequent increase in Aβ generation mediated via both decreased α-secretase activity (5) and increased γ-secretase activity, may be a mechanism whereby the apoE ε4 allele contributes to increased AD.

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