NFκB-dependent Control of BACE1 Promoter Transactivation by Aβ42*

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β-Amyloid (Aβ) peptides that accumulate in Alzheimer disease are generated from the β-amylloid precursor protein (βAPP) by cleavages by β-secretase BACE1 and by presenilin-dependent γ-secretase activities. Very few data document a putative cross-talk between these proteases and the regulatory mechanisms underlying such interaction. We show that presenilin deficiency lowers BACE1 maturation and affects both BACE1 activity and promoter transactivation. The specific γ-secretase inhibitor DFK167 triggers the decrease of BACE1 activity in wild-type but not in presenilin-deficient fibroblasts. This decrease is also elicited by catalytically inactive γ-secretase. The overexpression of APP intracellular domain (AICD), the γ/ε-secretase-derived C-terminal product of β-amylloid precursor protein, does not modulate BACE1 activity or promoter transactivation in fibroblasts and does not alter BACE1 expression in AICD transgenic brains of mice. A DFK167-sensitive increase of BACE1 activity is observed in cells overexpressing APPε (the N-terminal product of BAPP generated by ε-secretase cleavage harboring the Aβ domain but lacking the AICD sequence), suggesting that the production of Aβ could account for the modulation of BACE1. Accordingly, we show that HEK293 cells overexpressing wild-type βAPP exhibit a DFK167-sensitive increase in BACE1 promoter transactivation that is increased by the Aβ-potentiating Swedish mutation. This effect was mimicked by exogenous application of Aβ42 but not Aβ40 or by transient transfection of cDNA encoding Aβ42 sequence. The IkB kinase inhibitor BMS345541 prevents Aβ-induced BACE1 promoter transactivation suggesting that NFκB could mediate this Aβ-associated phenotype. Accordingly, the overexpression of wild-type or Swedish mutated βAPP does not modify the transactivation of BACE1 promoter constructs lacking NFκB-responsive element. Furthermore, APP/β-amylloid precursor protein-like protein deficiency does not affect BACE1 activity and expression. Overall, these data suggest that physiological levels of endogenous Aβ are not sufficient per se to modulate BACE1 promoter transactivation but that exacerbated Aβ production linked to wild-type or Swedish mutated βAPP overexpression modulates BACE1 promoter transactivation and activity via an NFκB-dependent pathway.

Alzheimer disease (AD) is characterized by abnormal deposition of a set of hydrophobic peptides called amyloid β (Aβ) peptides. The increase of cerebral Aβ levels is one of the common denominators characterizing both sporadic and familial forms of AD and therefore, if not demonstrated as the etiological cause of AD pathology, is often considered as a key factor contributing to the degenerative process (1). The mechanisms by which Aβ peptides are generated are a matter of intense research in the AD field. Aβ peptides are released from a transmembrane protein, β-amylloid precursor protein (βAPP), by the sequential attacks by β- and γ-secretase that liberate the N- and C-terminal moieties of Aβ peptides, respectively (2). All β-secretase-like activity appears to be borne by an aspartryl protease referred to as BACE1, ASP2, or memapsin 2 (3–7), whereas γ-secretase seems to be due to both presenilin (PS)-dependent and PS-independent activities (8–13).

A lot has been learned recently about the biology of BACE1. BACE1 is a type I transmembrane protein that undergoes several post-transduclational modifications. BACE1 is N-glycosylated in its ectodomain where six cysteine residues form intramolecular disulfide bridges (14). Immature glycosylated BACE1 rapidly undergoes a furin-mediated removal of its pro-domain (15) to generate the 70-kDa mature form of the enzyme. Mature BACE1 also undergoes palmitoylation at three cysteine residues and sulfation of N-glycosylated moieties (16). Of most importance, BACE1 depletion leads to full impairment of Aβ production and is apparently safe (17, 18). Thus, invalidated embryos are viable whereas adult BACE1−/− mice are fertile. Although recent data indicate that BACE1 could have other substrates besides βAPP, which could be involved in important myelination processes (19, 20), the above data identify BACE1 as a suitable therapeutic target.

PS belong to the high molecular weight γ-secretase complex, which also includes Aph-1, Pen-2, and nicastrin (21, 22). Besides their contribution to the intramembranous cleavage of βAPP, PS participate in the proteolytic activation/degradation

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* The abbreviations used are: AD, Alzheimer disease; PS, presenilins; βAPP, β-amylloid precursor protein; Aβ, β-amylloid peptide; AICD, APP intracellular domain; APLP2, β-amylloid precursor protein-like protein 2; CREB, cAMP-response element-binding protein; Tricine, N-(2-hydroxy-1,1-bis(hydroxymethyl)ethyl)glycine; NEP, nephrilysin.

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of a series of other substrates involved in many vital functions (23), thereby explaining the apparent remarkable pleiotropy of these proteins (24). Among other functions, PS contribute to cellular adhesion, cell death control, and cell signaling (23, 25, 26). Another remarkable property of PS concerns their ability to act as a molecular chaperone as was demonstrated for TrkB (27), telencephalin (28), and N-cadherin (29).

Of most interest, Hébert et al. (30) demonstrated by yeast two-hybrid assay, co-immunoprecipitation procedure, and pulldown experiments that BACE1 and PS1 physically interacted. More recently, Kuzuya et al. (31) established that PS1 could modulate BACE1 maturation. The above data agreed well with the demonstration that βAPP, PS1, and BACE1 co-localized in membrane vesicles that undergo axonal transport by a kinesin-dependent mechanism (32). However, the functional cross-talk between the two partners and the mechanisms by which PS could control BACE1 activity remained elusive. Here we show that PS control the promoter transactivation of BACE1, and we establish that this PS-dependent effect is related to its associated γ-secretase activity. Furthermore, we demonstrate that BACE1 activity and promoter transactivation were directly linked to exacerbated production of Aβ and unrelated to AICD generation. Finally, we establish that Aβ-mediated transactivation of BACE1 promoter involves the NFκB pathway.

MATERIALS AND METHODS

Cell Cultures and Transfections—Fibroblasts devoid of PS1 and PS2 and βAPP/APLP2 double knock-out fibroblasts have been described previously (33, 34). HEK293 overexpressing wild-type βAPP, Swedish mutated βAPP, APPe, wild-type PS1, and mutated AA-PS1 were obtained and cultured as described previously (35, 36). Transient transfections of 2 μg of total cDNA were carried out with Lipofectamine 2000 reagent (Invitrogen) according to previously reported procedures (37).

Transgenic Mouse Brain Tissue Preparations—Brains from Fe65 or Fe65/AICD transgenic mice (38) were homogenized with 10 mM Tris-HCl, pH 7.5, and lysed with a Dounce homogenizer (50 strokes). Samples were immediately assayed for β-secretase activity and analyzed for their endogenous BACE1 expression as described below (39).

Western Blot Analysis and Antibodies—BACE1, βAPP, APLP2, Tip60, ADAM10, and Fe65 were separated on 8% Tris/glycine gel acrylamide; PS were analyzed on 12% Tris/glycine gels as described previously (40). Proteins were transferred onto Hybond-C nitrocellulose membranes (Amersham Biosciences) and then probed overnight with the following appropriate antibodies: anti-BACE1 (Zymed Laboratories Inc.); anti-N terminus of PS1 (kindly provided by Dr. G. Thinakaran); 9E10 anti-Myc antibody to label AICDs (Aventis); anti-Fe65, anti-hemagglutinin (Tip60), 6E10 anti-Aβ, BR188 antibody that recognizes the C terminus of APP (provided by Dr. M. Goedert, Cambridge, UK); WO2 antibody (The Genetics Co., Zurich, Switzerland) recognizes 5–8 sequence of Aβ; 2H3 antibody (provided by Dr. D. Schenk, San Francisco) raised against 1–12 sequence of Aβ, 22C11 antibody (Roche Applied Science); anti-actin and anti-tubulin (Sigma); and anti-ADAM10 antibodies (Euromedex, Soufflémeyersheim, France). Immunological complexes were revealed with appropriate secondary antibody and detected using an electrochemiluminescence method with the Lumi-light Western blotting substrate (Roche Applied Science) as described previously (41).

BACE1 and ADAM10 Fluorimetric Assay—Cells were lysed with 10 mM Tris-HCl, pH 7.5, and then homogenates were monitored for their BACE1 activity as described previously (39, 42). Briefly, samples (30 μg of proteins diluted in 10 mM acetate buffer, pH 4.5) were incubated in a final volume of 100 μl of the above acetate buffer containing BACE1 substrate (10 μM, (7-methoxyxoumarin-4-yl)acetyl-SEVNLDAEFRK(2,4-dinitrophenyl)-RRNH2; R & D Systems) in the absence or presence of the previously described BACE1 inhibitor JMV2764 (50 μM) (39). BACE1 activity corresponds to the JMV2764-sensitive fluorescence recorded at 320 and 420 nm as excitation and emission wavelengths, respectively as described (39). ADAM10 activity was monitored as described previously (43).

Transactivation of BACE1 Promoter—Empty vector or cDNA encoding rat BACE1 promoter or deletion constructs in-frame with luciferase (44) were co-transfected with β-galactosidase (to normalize transfection efficiencies). HEK293 cells and fibroblasts devoid of presenilins were cultured in 12-well dishes or 60-mm-diameter dishes and transfected with 2 or 8 μg of the total amount of cDNA, respectively. For Aβ transfection experiments, cells were transfected with a total amount of 3 μg of cDNA encoding rat BACE1 promoter, β-galactosidase, and either empty vector or constructs harboring Aβ-(1–42), Aβ-(42-1), or Aβ-(1–40) (45). Thirty hours after transfection, cells were harvested with phosphate-buffered saline/EDTA (5 mM), pelleted by centrifugation (1000 × g, 5 min), lysed with 50 μl of lysis buffer (luciferase kit Promega), centrifuged for 5 min at 2000 rpm, and then luciferase activity was measured with 10 μl of supernatant and 50 μl of luciferase assay (Promega).

Effect of γ-Secretase Inhibitor Treatment on BACE1 Promoter Transactivation and Activity—Cells were treated for 16 h with various concentrations of the γ-secretase inhibitor DFK167 (46) or corresponding amounts of Me2SO (all DFK167 stocks were made at 10 mM in 100% Me2SO). Cells were harvested with phosphate-buffered saline/EDTA (5 mM), pelleted by centrifugation (1000 × g, 5 min), and lysed with Tris-HCl (10 mM, pH 7.5) or 50 μl of lysis buffer (luciferase kit Promega) to measure BACE1 activity and rat BACE1 promoter activity, respectively.

Effect of Exogenous Aβ on BACE1 Promoter Transactivation—Twenty four hours after transfection of rat BACE1 promoter, medium was replaced with Opti-MEM medium (Sigma) containing fetal bovine serum (2%) and complemented with phosphoramidon (10 μM) to prevent Aβ degradation. The cells were then treated for 48 h with various concentrations of synthetic Aβ42 or Aβ40 (Bachem).

Effect of Treatment with the IkB Kinase Inhibitor BMS345541—HEK293 overexpressing wild-type βAPP, Swedish mutated βAPP, or Aβ-treated cells were incubated for 18 h with the IkB kinase inhibitor BMS345541 (4(2′-aminoethyl)amino-1,8-dimethylimidazo(1,2-a)quinoxaline, 30 μM) or a corresponding amount of Me2SO in Opti-MEM medium containing 2% of fetal bovine serum.
mature form of the enzyme (Fig. 1). The identity of this protein remains still unknown, but we can rule out the possibility that this protein derives from abnormal prohormone convertase processing that would have occurred in the absence of PS because this protein remains totally insensitive to the convertase inhibitor α1-antitrypsin Portland variant (49, 50 and data not shown).

We examined whether PS deficiency-associated reduction of mature BACE1 expression was associated with a decrease of BACE1 enzymatic activity. Thus, the depletion of endogenous PS triggers a significant reduction of BACE1 activity (27.4 ± 4.8% compared with control, n = 6, p < 0.0001; see Fig. 1D). The similar decrease in both mature BACE1 expression and activity suggests that the above-described unidentified protein apparently does not harbor catalytic properties. Of most interest, we establish that fibroblasts devoid of PS display drastically lower BACE1 promoter transactivation (77.4 ± 7.4% compared with control, n = 9, p < 0.0001; see Fig. 1E). Overall, the above data suggested that PS modulate BACE1 activity by apparently controlling this protease at both transcriptional and post-transcriptional levels.

Presenilin-dependent γ-Secretase Activity Is Involved in the Control of BACE1—We examined whether the modulation of BACE1 activity could be linked to the enzymatic activity displayed by the PS-dependent γ-secretase complex. Two distinct sets of experiments suggest that it is indeed the case. First, we assessed the influence of a double mutation thought to yield a catalytically inactive PS1 (D257A/D385A-PS1, AA-PS1 (51)). Unlike wild-type PS1, which slightly increases BACE1 activity (Fig. 2B), AA-PS1 overexpression (Fig. 2A) reduces BACE1 activity (53.4 ± 8.2% compared with WT-PS1, n = 3, p < 0.01; Fig. 2B). Second, we assessed the influence of DFK167, a γ-secretase inhibitor that physically interacts with PS (52), on BACE1 activity. As a matter of controls, we first show that DFK167 does not directly interfere with BACE1 activity (Fig. 3A) but indeed abolishes Aβ production in HEK293 cells expressing Swedish mutated BAPP (Fig. 3B). Clearly, DFK167 reduces cellular BACE1 activity in wild-type fibroblasts (40.9 ± 3.6% for 10 μM DFK167 and 46.9 ± 4% for 50 μM DFK167 compared with control untreated cells, n = 16, p < 0.001; Fig. 3C) as well as BACE1 expression (Fig. 3C). However, the γ-secretase inhibitor neither alters BACE1 expression nor activity in PS-deficient cells (Fig. 3D). Altogether, both mutational and pharmacological data indicate that PS-induced modulation of BACE1 activity was apparently linked to PS-associated γ-secretase activity.

AICDs Do Not Modulate BACE1 Activity, in Vitro and in Vivo—Because the modulation of BACE1 was apparently linked to PS-dependent catalytic events, we postulated that the control of BACE1 should involve catabolites generated by PS-dependent γ-secretase-associated processes and harboring transcription factor properties. In this context, we examined whether the C-terminal products of BAPP hydrolysis could contribute to the modulation of BACE1 activity. Thus, these fragments called AICD (APP intracellular domain) have been shown to be derived from DFK167-sensitive γ- and ε-secretase proteolytic cleavages (53, 54) and to translocate into the nucleus (55, 56) where they participate in the transcription of several promoters (41, 57–60). AICDC59 (γ-secretase-derived) and AICDC50 (ε-secretase-derived) cDNAs were co-transfected with Fe65 and Tip60, two proteins reported to stabilize

Analysis of Aβ40 Production—Cells were allowed to secrete Aβ in Opti-MEM medium (Sigma) for 8 or 16 h in the presence of phosphoramidon (10 μM) (to prevent the degradation of secreted Aβ), in the absence or in the presence of DFK167 (50 μM), collected, supplemented with RIPA buffer (10 mM Tris-HCl, pH 8, EDTA 5 mM, NaCl 150 mM), and then incubated overnight with a 100-fold dilution of FCA18 or FCA3340 (47). Aβ40 was immunoprecipitated with protein A-Sepharose, analyzed on a 16.5% Tris/Tricine gel, Western blotted, and revealed with 6E10 as described previously (36).

Statistical Analysis—Statistical analysis was performed with Prism software (Graphpad, San Diego) using the Student-Newman-Keul’s multiple comparison test for one-way analysis of variance or unpaired t test for pairwise comparison.

RESULTS

Presenilin 1 and Presenilin 2 Deficiency Affects BACE1 Maturation, Activity, and Promoter Transactivation—We examined the influence of presenilins on the expression of BACE1. In wild-type fibroblasts, pro-BACE1 and mature BACE1 were detected at their previously reported molecular weights (Fig. 1A) (48). Interestingly, the depletion of both PS1 and PS2 lowers the level of mature BACE1 (44.4 ± 10.1% compared with control, n = 6, p < 0.01) without affecting pro-BACE1 (Fig. 1B and C). In PS-deficient fibroblasts, an additional band harboring BACE-1-like immunoreactivity was detected above the mature form of the enzyme (Fig. 1A). The identity of this protein is still unknown, but we can rule out the possibility that this protein derives from abnormal prohormone convertase proc-
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AICD and favor their nuclear translocation (57). In these conditions, AICDs are readily detectable in both PS+/+ and PS−/− fibroblasts (Fig. 4A). As shown in Fig. 1, in this independent set of experiments, both BACE1 expression (31% reduction when compared with control) and activity (compare empty bars) are reduced in PS−/− fibroblasts (Fig. 4B). However, AICDC59 and AICDC50 are unable to affect BACE1 activity in both wild-type and PS-deficient fibroblasts (Fig. 4B) and do not modify BACE1 promoter transactivation (Fig. 5). It should be noted that the transfection of cDNA coding for E-CTF2, ALID1, and ALID2, the γ-secretase-derived intracellular fragments of E-cadherin, APLP1, and APLP2, respectively, did not increase BACE1 expression, activity, and promoter transactivation (data not shown). These data were confirmed in vivo. Thus, we examined the expression and activity of BACE1 in single Fe65 and double Fe65/AICD transgenic mice brains. As described previously (61), AICD-like immunoreactivity is clearly observed in double transgenic mice brain (Fig. 6A), whereas endogenous levels of cerebral AICD are poorly detectable, in agreement with the low catabolic stability reported for these fragments (62). However, the overexpression of AICD does not modify BACE1 expression (Fig. 6B) and activity (Fig. 6D) in these transgenic mice brains.

APPε-associated and DFK167-sensitive Modulation of BACE1 Activity—In search of another plausible γ-secretase-derived product able to modulate BACE1 but distinct of AICDs, we examined the influence of APPε on the activity of BACE1. APPε corresponds to the N-terminal βAPP fragment generated by ε-secretase and therefore lacks the C-terminal AICD domain (36). We established previously that APPε undergoes both β-secretase and DFK167-sensitive γ-secretase cleavages (36), yielding the Aβ. Therefore, cells overexpressing APPε (Fig. 7A) could be seen as a theoretically useful model to examine whether the PS-dependent γ-secretase-mediated control of BACE1 was linked to Aβ production. First, we show that the transient transfection of either wild-type βAPP or APPε cDNA in HEK293 cells, which enhances Aβ recovery (Fig. 7A), triggers a similar increase in BACE1 promoter transactivation (Fig. 7B). Second, in stably transfected APPε-expressing HEK293 cells (Fig. 7C), we observed a DFK167-sensitive reduction of BACE1 activity (29.8 ± 7.9%) compared with untreated APPε cells, n = 12, p < 0.001; Fig. 7D). Overall, these data indicate that Aβ but not AICD contributes to the modulation of BACE1 activity.

Aβ Controls BACE1 Promoter Transactivation but Does Not Affect α-Secretase Activity—We examined whether Aβ-associated control of BACE1 activity could be directly linked to the level of Aβ. In this context, we took advantage of cells lines overexpressing wild-type (WT-APP) or Swedish-mutated
The transcription and translation of AICD cDNA was monitored by Western blotting. The expression of AICD was confirmed by the presence of a specific band corresponding to the expected size. The results are shown in Figure 4, which displays the Western blot images of HEK293 cells transfected with either empty pcDNA3 or AICD cDNA. The bars indicate the relative expression levels of AICD, and the y-axis represents the percent of control, with ns indicating no significant difference.

Figure 5 shows the effect of AICD on BACE1 expression and activity in wild-type fibroblasts. The AICD expression was measured by luciferase assay, and the results are shown in the graph. The bars indicate the percent of control, and the y-axis represents the percent of that observed in mock-transfected PS-positive fibroblasts. The p-values are denoted as ns, indicating no significant difference.

Figure 6 illustrates the endogenous BACE1 expression and activity in Fe65-AICD transgenic mice brains. The expression and activity were measured by Western blotting and luciferase assay, respectively. The bars indicate the percent of control, and the y-axis represents the percent of that observed in mock-transfected PS-positive mice brains. The p-values are denoted as ns, indicating no significant difference.

Figure 7 shows the influence of APP on BACE1 activity and promoter transactivation. A mock-transfected HEK293 cells were transfected with empty pcDNA3, wild-type βAPP (WT-APP), or APP cDNAs. Secrete Aβ was monitored by immunoprecipitation with FC3340 antibody and analyzed after 16.5% Tris/Tricine electrophoresis. The bars indicate the percent of control, and the y-axis represents the percent of that observed in mock-transfected PS-positive cells. The p-values are denoted as ns, indicating no significant difference.

In conclusion, the transcription and translation of AICD cDNA were confirmed by Western blotting. The expression of AICD was measured by luciferase assay, and the endogenous BACE1 expression and activity in Fe65-AICD transgenic mice brains were monitored. The influence of APP on BACE1 activity and promoter transactivation was investigated, and no significant differences were observed.
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**FIGURE 8. Influence of wild-type and Swedish-mutated APP on BACE1 promoter transactivation.** A, HEK stably overexpressing empty vector (Ct), WT-βAPP (WT-APP), or Swedish-mutated βAPP (Swe-APP) were incubated for 16 h with DFK167 (50 μM) or an equal amount of Me₂SO (DMSO), and then secreted Aβ was monitored after immunoprecipitation with FCA18 antibody and analyzed after 16.5% Tris/Tricine electrophoresis and Western blot with 6E10 antibody as described under “Materials and Methods.” Note that long term exposure (dark) allows detecting Aβ in mock-transfected (Ct) cells. βAPP-like immunoreactivity was monitored in the indicated cell homogenates with 22C11 antibody as described under “Materials and Methods.” B, HEK293 cells overexpressing WT-APP or Swe-APP (B) were transiently transfected with rat BACE1 promoter-luciferase cDNA as described under “Materials and Methods.” Thirty hours after transfection, cells were treated for 16 h with DFK167 (50 μM) or adequate amount of Me₂SO, and then luciferase reporter activity was measured as described under “Materials and Methods.” Bars correspond to luciferase activity expressed as percent of that observed in control untreated cells (Ct, Ct) taken as 100 or cells transfected with empty vector (pTet, taken as 100) and are the means ± S.E. of eight independent determinations. *, p < 0.05; **, p < 0.01; ns not statistically significant.

At Asp-1 residue (47), we show that Swe-APP cells produce more Aβ than WT-APP cells (Fig. 8A). As expected, this effect was drastically reduced by DFK167 (Fig. 8A, dark). It should be noted here that prolonged exposure of the gels allows revealing DFK167-sensitive production of Aβ in mock-transfected cells (Fig. 8A). Interestingly, WT-APP expression increases BACE1 promoter transactivation (compare Ct and WT-APP in Fig. 8B), a phenotype that was further accentuated in cells overexpressing Swe-APP (compare WT-APP and Swe-APP in Fig. 8B). DFK167 significantly reduced but did not abolish APP-associated increase of BACE1 promoter transactivation (Fig. 8B).

APPɛ-, WT-APP-, and Swe-APP-associated modulation of BACE1 promoter transactivation strongly suggested that Aβ was indeed the molecular determinant responsible for this phenotype. Two direct lines of evidence directly reinforced this view. First, exogenous application of Aβ42 (Fig. 9A) but not Aβ40 (Fig. 9B) triggers the transactivation of the BACE1 promoter. Second, the transient transfection of DNA constructs harboring the Aβ-(1–42) sequence (45) also increases BACE1 promoter transactivation, whereas vectors coding for the “reverse peptide” Aβ-(42–1) or for Aβ-(1–40) remained biologically inert on this paradigm (Fig. 9C). Therefore, we can definitely conclude that Aβ42 acts as a modulator of BACE1 transactivation.

To establish whether Aβ could also influence other activities involved in the processing of βAPP, we examined the influence of Aβ levels on the α-secretase pathway. We show that ADAM10 expression (Fig. 10A) and whole α-secretase-like activity (Fig. 10, B and C) remained unaffected by the overexpression of either WT- or Swe-APP.

**FIGURE 9. Exogenous or transfected Aβ42 but not Aβ40 increase BACE1 promoter transactivation.** A and B, HEK293 cells were transiently transfected with rat BACE1 promoter luciferase DNA as described under “Materials and Methods.” Twenty-four hours after transfection, cells were exogenously treated for 48 h with increasing concentrations of synthetic Aβ42 (A) or Aβ40 (B), and then luciferase reporter activity was measured as described under “Materials and Methods.” C, HEK293 cells were transiently transfected with rat BACE1 promoter luciferase DNA and either empty vector or constructs encoding Aβ-(1–42), Aβ-(42–1), and Aβ-(1–40) as described under “Materials and Methods.” Thirty hours after transfection, luciferase reporter activity was measured as described under “Materials and Methods.” Bars correspond to luciferase activity expressed as percent of that observed in control untreated cells (A, Ct) taken as 100 or cells transfected with empty vector (pTet, taken as 100) and are the means ± S.E. of eight independent determinations. *, p < 0.05; **, p < 0.01; ns not statistically significant.

Aβ-induced Regulation of BACE1 Promoter Transactivation Is NFκB-dependent—Several lines of evidence indicated that BACE1 promoter activity was regulated by the transcription factor NFκB (66). It was therefore tempting to speculate on the involvement of the NFκB pathway in the Aβ-associated control of BACE1. NFκB could be indirectly targeted by BMS345541, a selective blocker of IκB kinases 1 and 2 (67), thereby leading to NFκB inactivation. However, we establish, in agreement with a previous study documenting the influence of a series of NFκB inhibitors (68), that BMS345541 indeed blocks NFκB transcriptional activity (our data not shown) and also inhibits the production of both Aβ40 and Aβ42 by Swe-APP-expressing cells (Fig. 11A). Therefore, the use of BMS345541 on cells would not allow discriminating between a direct effect of the inhibitor on NFκB-dependent transactivation of BACE1 promoter and an indirect effect because of an upstream inhibition of Aβ production. To circumvent this problem, we examined the effect of BMS345541 on BACE1 modulation by exogenous application
**Figure 10.** Overexpression of wild-type and Swedish-mutated APP does not affect ADAM10 expression and α-secretase-like activity. A. Western blot analysis of ADAM10 was performed on HEK293 cells overexpressing empty vector (Ct), WT-APP (WT-APP) or Swedish-mutated APP (Swe-APP) as described under “Materials and Methods.” ADAM10 antibody revealed both immature (pro-ADAM10) and mature (mat-ADAM10) ADAM10. B, α-secretase activity was fluorimetrically recorded with JMV2770 (10 μM) on intact HEK293 cells stably overexpressing empty vector (Ct) wild-type (WT-APP), or Swedish-mutated APP (Swe-APP), for the indicated times, in the absence or in presence of o-phenanthroline (100 μM) as described under “Materials and Methods.” C. α-secretase specific activity corresponds to the o-phenanthroline-sensitive fluorescence (arbitrary units (AU) per mg of proteins). Bars are the means ± S.E. of six independent experiments. *, p < 0.01; **, p < 0.001.

**Figure 11.** The IKK inhibitor BMS345541 decreases Aβ recovery and reverses the increase of BACE1 promoter transactivation triggered by Aβ42 application or WT-APP overexpression. A. HEK293 cells overexpressing Swedish-mutated APP (Swe-APP) were incubated for 18 h with the IKK inhibitor BMS345541 (30 μM) or equal amounts of Me2SO (DMSO), and then secreted Aβ was monitored after immunoprecipitation with FCA18 antibody and analyzed after 16.5% Tris/Tricine electrophoresis and Western blot with 6E10 antibody as described under “Materials and Methods.” Bars in A correspond to the densitometric analysis of secreted Aβ immunoreactivity expressed as that observed in Me2SO-treated cells (taken as 100) and are the means ± S.E. of six independent experiments. *, p < 0.0001. B and C, mock-transfected (B) or WT-APP-expressing (C) HEK293 cells were transiently transfected with the indicated rat BACE1 promoter-luciferase constructs as described under “Materials and Methods.” Thirty hours after transfection, luciferase reporter activity was measured as described under “Materials and Methods.” Bars correspond to luciferase activity expressed as percent of that observed in mock-transfected cells transiently transfected with the entire rat BACE1 promoter construct (taken as 100) and are the means ± S.E. of eight independent determinations. *, p < 0.01; **, p < 0.001, ns, not statistically significant.

**Figure 12.** Influence of wild-type and Swedish-mutated APP on full-length and deleted BACE1 promoter transactivation. A, a schematic map of the deletion mutant constructs. B, mock-transfected HEK293 cells (Ct) or cells overexpressing wild-type APP (WT-APP) or Swedish-mutated APP (Swe-APP) were transiently transfected with the indicated rat BACE1 promoter-luciferase constructs as described under “Materials and Methods.” Thirty hours after transfection, luciferase reporter activity was measured as described under "Materials and Methods.” Bars correspond to luciferase activity expressed as percent of that observed in mock-transfected cells transiently transfected with the entire rat BACE1 promoter construct (taken as 100) and are the means ± S.E. of six independent determinations. *, p < 0.01; **, p < 0.001. of Aβ42. As described in Fig. 9A, in this independent set of experiments, Aβ42 triggered an increased BACE1 promoter transactivation (Fig. 11B). Interestingly, this appears to be prevented by BMS345541 (Fig. 11B). Accordingly, BMS345541 drastically reduces the increase of BACE1 promoter transactivation triggered by WT-APP overexpression (Fig. 11C). These data strongly suggest that BMS345541 triggers its effect downstream to Aβ production and that the NFκB pathway mediates the Aβ-induced modification of BACE1 transactivation. To reinforce this hypothesis, we examined the ability of cell lines expressing various levels of Aβ to transactivate promoter constructs of BACE1, some of which were deleted of NFκB-responsive elements (Fig. 12A). As expected, the overexpression of WT-APP or Swe-APP increases the transactivation of the full-length promoter of BACE1 (Fig. 12B). Interestingly, the 3’ deletion of a domain, including the NFκB-responsive element (BPR-Nco), drastically reduces BACE1 activation (Fig. 12B), whereas the shortest 3’ construct (BPR-Avr) and 5’-deleted construct (1–2 Del) were not transactivated in these cells (Fig. 12B). It should be noted that the transcription of BPR-Nco was also reduced in mock-transfected cells. Together with the fact that BMS345541 also reduces BACE1 promoter transactivation in mock-transfected cells (Fig. 11, B and C), this could indicate that endogenous NFκB participates in the control of BACE1 besides its role in Aβ-mediated phenotype. Overall, our data firmly suggest that Aβ-mediated control of BACE1 involves NFκB-associated transactivation of the BACE1 promoter.
Endogenous Aβ Does Not Modify BACE1 Expression—It is noteworthy that DFK167 did not modify BACE1 promoter transactivation in the Mock-transfected HEK293 cells (Fig. 8B). This suggested that the low levels of endogenous Aβ in these cells (Fig. 8A) would not be sufficient to alter BACE1 transactivation. To confirm this hypothesis, we examined the status of BACE1 expression and activity in double knock-out βAPP/APLP2 fibroblasts. These cells were chosen because they do not produce Aβ, AICD, or AICD-like (ALIDs) fragments. We show that the lack of these proteins does not alter BACE1 expression (Fig. 13, A and B) or activity (Fig. 13C). Overall, our data indicate BACE1 is not controlled by endogenous AICD or Aβ production and that the modulation of BACE1 could be triggered by elevated levels of Aβ.

DISCUSSION

One of the canonical hallmarks in Alzheimer disease-affected brain is the widespread distribution of extracellular deposits called senile plaques. A network of evidence based on biochemical, biological, anatomical, and genetic approaches indicates that amyloid β-peptides, the main component of senile plaques, play a central role in AD etiology (69). As a key argument to support this hypothesis is the observation that most, if not all, mutations responsible for aggressive and early onset AD cases have in common the ability to trigger modifications of the levels, the nature, or the biophysical properties of the Aβ peptides generated (for reviews see Refs. 2, 24). The definitive identification of the cellular and molecular mechanisms underlying such alterations is still lacking. One of the likely hypotheses would be that γ-secretase activity could be enhanced in AD, thereby increasing Aβ production. However, the demonstration of an up-regulation of γ-secretase associated with sporadic AD remains to be established. Furthermore, whether the alterations triggered by pathogenic mutations also account for those responsible for sporadic AD cases remains doubtful. It is more generally admitted that mutations modify γ-secretase-mediated cleavage specificity rather than alter its catalytic efficiency. Thus, mutations located close to the C terminus of Aβ apparently modify the ratio of AβX-42 over AβX-40 rather than the total Aβ load. Conversely, the Swedish mutation located downstream to the Aβ N terminus triggers an overload of Aβ40. Therefore, although the overall alteration of Aβ production could be seen as a common denominator between sporadic and genetic cases of AD, close inspection reveals specific molecular dysfunctions.

Another way of modifying cellular Aβ homeostasis could occur downstream to Aβ production. Neprilysin (NEP) and insulin-degrading enzyme are the two major putative metalloproteases involved in Aβ catabolism (70–72). NEP was shown to display higher catalytic efficiency toward Aβ40 versus Aβ42 (73). Therefore, early deterioration observed in those cases of familial AD where Aβ42 production is more specifically altered could be due to NEP-mediated exacerbation of the Aβ42 over Aβ40 ratio. On the other hand, it has been documented that NEP and insulin-degrading enzyme display age-related decrease in their cerebral levels (74). This observation could account for the late increase in Aβ levels observed in sporadic cases of AD.

Here we show that PS deficiency decreases the activity and promoter transactivation of the β-secretase BACE1. As described previously (31), we show that part of the modulation of BACE1 activity could be accounted for by a PS-dependent defect in BACE1 maturation. However, two lines of data demonstrate that PS-dependent γ-secretase activity could also contribute to the control of Aβ production. First, the overexpression of a catalytically inactive PS1 mutant lowers BACE1 activity. Second, the γ-secretase inhibitor DFK167 lowers BACE1 expression and activity in wild-type but not in PS-deficient fibroblasts.

The above data prompted us to examine which putative product generated by PS-dependent γ-secretase could harbor the potential of modulating BACE1. We first focused on AICD, the γ-secretase-derived intracellular fragment of βAPP because this product displays transcription factors properties that could therefore account for PS-dependent enhancement of BACE1 promoter transactivation. Even in conditions where AICD translocation and stability are favored (i.e. after co-expression with Tip60 and Fe65), we were unable to demonstrate AICD-associated increase in BACE1 activity in wild-type and PS-deficient fibroblasts. That AICD was biologically inert on BACE1 was confirmed in vivo, in double transgenic mice overexpressing both AICD and Fe65 (38), a model where neprilysin (61) and p53 (39) had been shown to be up-regulated. These data were corroborated by our observation that transient transfection of wild-type βAPP and APPe, the e-secretase-derived N-terminal fragment of βAPP lacking AICD, both trigger similar increase of BACE1 promoter transactivation.

4 J. Dunys, C. Alves da Costa, and F. Checler, unpublished data.
Several lines of data support the view that Aβ itself accounts for the observed γ-secretase-mediated effect on BACE1. First, BACE1 activity is inhibited by DFK167 in APP-expressing cells. Second, the comparison of stably transfected HEK293 cells overexpressing either wild-type or Swedish mutated βAPP indicates that the mutation that further enhances Aβ production also potentiates the βAPP-associated increase of BACE1 promoter transactivation. Third, exogenous application of Aβ42 but not Aβ40 increases both promoter transactivation of BACE1. Fourth, transient transfection of cDNA encoding Aβ(1–42) but not Aβ(42–1) or Aβ(1–40) also trigger increased BACE1 promoter activity.

The above observation is of major importance because this suggests that the control of BACE1 is intrinsically linked to the level of Aβ42. Indeed, several lines of evidence indicate that normal physiological amounts of Aβ do not influence BACE1. First, although mock-transfected HEK293 produce low but measurable DFK167-sensitive Aβ, this inhibitor did not modulate BACE1 promoter transactivation in these cells. Second, BACE1 expression and activity were similar in wild-type and βAPP/APP2-deficient fibroblasts, which do not produce endogenous levels of Aβ. The above observations suggest that the control of BACE1 by Aβ only takes place when endogenous concentrations of the peptide increase significantly. This also suggests that the decrease of BACE1 activity observed in PS-deficient fibroblasts was not because of endogenous Aβ dephosphorylation but more likely attributable to an AICD-independent γ-secretase-mediated product that remains to be identified. One can postulate that this apparent concentration-dependent Aβ-mediated effect is because of the propensity of this peptide to aggregate when reaching the threshold of its solubility because several studies have clearly demonstrated that Aβ fibrils are the toxic species (75). It is tempting to speculate that this could reflect the dysfunction occurring in sporadic cases of AD.

The question arises as to whether Aβ either directly modulates BACE1 promoter transactivation, indirectly targets an intermediate cellular modulator of BACE1 transactivation, or if both direct and indirect mechanisms could take place. We have identified NFKB as a molecular intermediate involved in the Aβ-mediated control of BACE1. First, the inhibitor of 1kB kinase that blocks NFKB transcriptional activity fully reverses the Aβ42-induced increase of BACE1 promoter transactivation. Second, deletions that disrupt an NFKB-responsive element in the promoter of BACE1 abolish the ability of WT-APP and Swedish-APP cells to control BACE1 promoter transactivation. These data agree well with a previous study showing that BACE1 promoter transactivation could be regulated by NFKB in neuronal cells (66).

It is noteworthy that the dose of exogenous Aβ able to trigger BACE1 promoter activation was a priori high but in the range of those used in a previous study aimed at comparing BACE1 promoter activity in neurons and glial cells (66). These high doses likely do not reflect the real effective dose because Aβ treatment requires long incubation times (see “Materials and Methods”) during which several parameters, including degradation processes, bioavailability, and intracellular accessibility, can not be controlled. Furthermore, several lines of evidence indicate that the effect triggered by exogenous Aβ42 cannot be seen as artifactual. First, the same dose of Aβ40 remains biologically inert on BACE1 promoter activation. Second, in transfection experiments similar to those described in Ref. 45, Aβ(1–42) but not Aβ(42–1) or Aβ(1–40) mimic the phenotype triggered by exogenous Aβ(1–42), although the Aβ levels were drastically lower in transfection experiments. Third, both exogenous application of Aβ42 and expression of wild-type or Swedish-mutated APP activate BACE1 promoter in a BMS345541-sensitive manner.

It should be noted that the disruption of the NFKB-responsive element did not totally reverse WT-APP- and Swedish-APP-associated increase of BACE1 promoter activity (see Fig. 12B), suggesting that additional mechanisms may take place. Thus, BACE1 promoter harbors a canonical CREBbinding site that appears to act as a repressor of BACE1 transactivation because mutation at this site increases reporter protein expression (76). It is noteworthy that Aβ has been previously shown to down-regulate CREB-induced signaling in cultured neurons (77), apparently by blocking the nuclear translocation of phosphorylated CREB (78). Of most interest, this process appears dependent on high intracellular Aβ concentrations (78). Therefore, Aβ-induced lowering of CREB-associated pathway could ultimately lead to abolishing CREB-induced repression of BACE1, thereby increasing transcription of its promoter.

Besides such possible indirect modulation of BACE1 by Aβ, one should also underline that several studies reported on a genuine role of Aβ as transcription factor. Thus, Ohyagi et al. (45) demonstrated that Aβ directly binds to elements of the p53 promoter. Interestingly, the Aβ binding sequence found in the p53 promoter resembles some sequence domains found on the BACE1 promoter. In agreement with this observation, Maloney et al. (79) showed that these sequences bind Aβ in vitro and influence BACE1 promoter constructs transactivation in cells.

Overall, our work identifies Aβ42 as a regulator of BACE1 and suggests that only abnormally high levels of this pathogenic species of Aβ could influence BACE1 promoter transactivation, at least in part via the NFKB pathway. This vicious circle by which pathological production of Aβ42 feeds its own production could contribute to both sporadic and genetic cases of Alzheimer disease.

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