Partial Reduction of BACE1 Has Dramatic Effects on Alzheimer Plaque and Synaptic Pathology in APP Transgenic Mice

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The aspartyl protease β-site amyloid precursor protein cleaving enzyme 1 (BACE1) initiates processing of amyloid precursor protein (APP) into amyloid β (Aβ) peptide, the major component of Alzheimer disease (AD) plaques. To determine the role that BACE1 plays in the development of Aβ-driven AD-like pathology, we have crossed PDAPP mice, a transgenic mouse model of AD overexpressing human mutated APP, onto mice with either a homozygous or heterozygous BACE1 gene knock-out. Analysis of PDAPP/BACE1(−/−) mice demonstrated that BACE1 is absolutely required for both Aβ generation and the development of age-associated plaque pathology. Furthermore, synaptic deficits, a neurodegenerative pathology characteristic of AD, were also reversed in the bigenic mice. To determine the extent of BACE1 reduction required to significantly inhibit pathology, PDAPP mice having a heterozygous BACE1 gene knock-out were evaluated for Aβ generation and for the development of pathology. Although the 50% reduction in BACE1 enzyme levels caused only a 12% decrease in Aβ levels in young mice, it nonetheless resulted in a dramatic reduction in Aβ plaques, neuritic burden, and synaptic deficits in older mice. Quantitative analyses indicate that brain Aβ levels in young APP transgenic mice are not the sole determinant for the changes in plaque pathology mediated by reduced BACE1. These observations demonstrate that partial reductions of BACE1 enzyme activity and concomitant Aβ levels lead to dramatic inhibition of Aβ-driven AD-like pathology, making BACE1 an excellent target for therapeutic intervention in AD.

Alzheimer disease is the major cause of dementia in elderly people and is characterized by progressive cognitive decline. There is no cure, current treatments offer only temporary relief, and death invariably ensues. Substantial evidence suggests that the amyloid β peptide (Aβ)6 is the cause of Alzheimer disease (AD)-associated neuropathology (1). Aβ is derived by sequential proteolysis of the amyloid precursor protein (APP) through β- and γ-secretase activities and is widely deposited in amyloid plaques in the brains of individuals with AD (2, 3). Therefore, inhibiting the action of one or both of these enzymatic activities may provide inaugural disease-modifying therapies for AD.

The aspartyl protease BACE1 is the primary β-secretase (4–6) and is the sole β-secretase in mice, since its genetic ablation fully abolishes Aβ generation (7–9). Early reports indicated that BACE1 knock-out animals are healthy and fertile, with no histological pathologies, suggesting that inhibition of BACE1 for therapeutic intervention in AD would have no mechanism related toxicities (7, 9, 10). In contrast, recent reports of partially penetrant lethality and cognitive deficits in BACE1 knock-out animals do suggest potential liabilities of complete BACE1 inhibition (11, 12). As the initiating enzyme in the generation of Aβ, BACE1 is a key drug target and would be predicted to abrogate pathologies associated with any form of Aβ. To avoid potential side effects resulting from complete loss of BACE1 activity, it is critical to determine the required degree of inhibition necessary for potential therapeutic benefit.

Cognitive decline in AD is believed to be due to the progressive degeneration of synapses and neurons (13, 14), yet the precise relationship between Aβ plaques, and neurodegeneration is still unclear. Transgenic mice that neuronally overexpress human APP (hAPP) carrying mutations associated with familial-inherited forms of AD, such as the PDAPP mouse, develop several AD-like neuropathologies including amyloid plaques and synaptic deficits (15–20). BACE1 gene ablation led to reduced plaque pathology and behavioral deficits in hAPP transgenic mice (10, 11, 21, 22). However, the complete characterization of age-dependent, AD-related neuropathology and neurodegeneration in human APP transgenic mice affected by BACE1 knock-out is still lacking. In particular, the role of Aβ in

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synaptic pathology, a plaque-independent pathology (14, 23), and a robust correlate of cognitive decline in AD awaits further confirmation.

We have used BACE1 knock-out animals crossed with PDAPP mice to define the role of Aβ in plaque, neuritic, and synaptic pathology and, thus, evaluate BACE1 inhibition as a therapeutic approach for AD. To determine the degree of BACE1 inhibition required to impact pathology, we crossed PDAPP mice onto the heterozygous BACE1 knock-out background and examined the effects of partially reduced BACE1 on Aβ levels and on AD-like pathologies. We find that homoygous ablation of BACE1 reduces plaque and synaptic pathologies in the PDAPP mouse model. Ablation of a single BACE1 allele has only a modest effect on Aβ levels yet significantly reduces plaque and synaptic pathologies in these mice. These observations suggest that only modest inhibition of Aβ in AD patients could lead to a significant reduction of pathology.

EXPERIMENTAL PROCEDURES

Animals—Table 1 shows all the genotypes and their abbreviations for the mice analyzed in this study. Mouse experiments were performed in accordance with Institutional Animal Care and Use Committee policies and procedures. WT/BACE1(+/−) mice, with exon 1 (exon containing the initiating ATG) deleted as described previously (7), were crossed onto PDAPPHom/BACE1(+/+) mice from line 109 (16, 24) to generate PDAPP/BACE1(+/+) and PDAPP/BACE1(+/-) animals. WT/BACE1(+/-) animals were generated on a 129sw/Ola strain ES cell and bred for three subse-quent generations onto 129sw antibody, specific for cleaved neo-epitope of APP-Swe. The cleaved product was measured by ELISA using anti-bacterial maltose-binding protein fused to the C-terminal 125-amino acid sequence of the Swedish variant of APP (APP-Swe). The cleaved product was measured by ELISA using anti-bacterial maltose-binding protein fused to the C-terminal 125-amino acid sequence of the Swedish variant of APP (APP-Swe). The cleaved product was measured by ELISA using anti-bacterial maltose-binding protein fused to the C-terminal 125-amino acid sequence of the Swedish variant of APP (APP-Swe).

Plaque burden and neuritic dystrophy were assessed by quantitative immuno-peroxidase histochemistry on free-floating, 40-μm-thick vibratome sections using the monoclonal anti-Aβ antibody 8E5 for the detection of neuritic dystrophy as described (26, 27). For each of these markers, six immunolabeled sections were analyzed per mouse, and the average of the individual measurements was used to calculate group medians.

Quantitative Synaptophysin Immunohistochemistry—Forty-μm-thick free-floating sections were immunostained with 1:850-diluted anti-SYN antibody (Clone SY38, Dako, Carpen-teria, CA) and fluorescein isothiocyanate-labeled secondary antibody following a standard protocol. Immunolabeled brain sections were assigned code numbers to ensure objective assessment and imaged with a Bio-Rad MRC-1024 laser-scanning confocal microscope mounted on a Nikon Optiphot-2 microscope with Lasersharp software as described (28, 29). Synaptophysin levels were assessed in the frontal neocortex and the hippocampal outer molecular layer (OML) in two sections/animal (29). For each mouse we obtained four confocal images (two per section) of the neocortex and two confocal images (one per section) of the hippocampal OML, each covering an area of 240 μm × 180 μm. The iris and gain levels were adjusted to obtain images with a pixel intensity within a linear range. Digitized, 8-bit images were transferred to a Macintosh computer, and the average pixel intensity of synaptophysin staining was calculated for each image with NIH Image. This approach for the assessment of synaptic degeneration has been validated in various experimental models of neurodegeneration (23, 29) and in diseased human brains (28).

Statistical Analyses—Parametric data were analyzed by one-way analysis of variance followed by Dunnett’s test, or Tukey multiple comparison tests where appropriate. Non-parametric data were analyzed by Mann-Whitney or Kruskall-Wallis’ test followed by Dunn’s test for comparison of multiple data sets. A p < 0.05 was considered significant. All analyses were done with the Prism software (GraphPad, San Diego, CA).

### Table 1: List of abbreviations for transgene genotypes

| PDAPP transgene | BACE1 gene | Abbreviation |
|----------------|------------|--------------|
| Wild type      | Wild type  | WT/BACE1(+/-)|
| Heterozygous   | Wild type  | PDAPP/BACE1(+/-)|
| Wild type      | Complete knockout | WT/BACE1(-/-) |
| Heterozygous   | Partial knockout | PDAPP/BACE1(-/-) |
| Homozygous     | Partial knockout | PDAPP/BACE1(+/-) |
| Homozygous     | Complete knockout | PDAPP/BACE1(-/-) |

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(Aβ1–42), and of Aβ-42 were quantitated by ELISA as described (25). The total Aβ (Aβ1–42) sandwich ELISA consists of the capture antibody 266, which is specific to amino acids 13–28 of Aβ, and the biotinylated reporter antibody 3D6, which is specific to amino acids 1–5 of Aβ. The plasma Aβ ELISA assay was performed as described (25) except Aβ standards, samples, and biotinylated antibody are diluted in specimen diluent with protease inhibitors for the assay.

β-Secrate activity was measured from crude membrane homogenates of hemibrains as described (7). P2 membranes were prepared from brain hemisections and extracted with buffer containing 0.2% Triton X-100. All assays contained 10 μg of membrane protein per ml. The substrate used was recombinant bacterial maltose-binding protein fused to the C-terminal 125-amino acid sequence of the Swedish variant of APP (APP-Swe). The cleaved product was measured by ELISA using anti-bacterial maltose-binding protein captured and detected with 129sw antibody, specific for cleaved neo-epitope of β-secreted APP-Swe.

Plaque burden and neuritic dystrophy were assessed by quantitative immuno-peroxidase histochemistry on free-floating, 40-μm-thick vibratome sections using the monoclonal anti-Aβ antibody 8E5 for the detection of plaques or the human specific APP antibody 8E5 for the detection of neuritic dystrophy as described (26, 27). For each of these markers, six immunolabeled sections were analyzed per mouse, and the average of the individual measurements was used to calculate group medians.

Quantitative Synaptophysin Immunohistochemistry—Forty-μm-thick free-floating sections were immunostained with 1:850-diluted anti-SYN antibody (Clone SY38, Dako, Carpenteria, CA) and fluorescein isothiocyanate-labeled secondary antibody following a standard protocol. Immunolabeled brain sections were assigned code numbers to ensure objective assessment and imaged with a Bio-Rad MRC-1024 laser-scanning confocal microscope mounted on a Nikon Optiphot-2 microscope with Lasersharp software as described (28, 29). Synaptophysin levels were assessed in the frontal neocortex and the hippocampal outer molecular layer (OML) in two sections/animal (29). For each mouse we obtained four confocal images (two per section) of the neocortex and two confocal images (one per section) of the hippocampal OML, each covering an area of 240 μm × 180 μm. The iris and gain levels were adjusted to obtain images with a pixel intensity within a linear range. Digitized, 8-bit images were transferred to a Macintosh computer, and the average pixel intensity of synaptophysin staining was calculated for each image with NIH Image. This approach for the assessment of synaptic degeneration has been validated in various experimental models of neurodegeneration (23, 29) and in diseased human brains (28).

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RESULTS

Elimination of Cerebral Aβ Blocks the Development of Plaque Pathology in PDAPP Mice—We and others (7–9) have shown that complete BACE1 gene knock-out mice do not produce Aβ. Fig. 1A shows that this is also true for Aβ produced from the human APP transgene in the cortex of young PDAPP mice. Although robust levels of Aβ were detected by ELISA in the cortex of PDAPP/BACE(+/+) animals, none was detectable in cortex of PDAPP/BACE(−/−) mice. This allowed us to dissect which pathological features of the PDAPP animals are due to Aβ and which are due to potential other effects of the APP transgene.

Luo et al. (10) have reported that BACE1 knock-out prevented the development of amyloid plaque in Tg2576 APP transgenic mice. Because PDAPP (line 109) mice develop more aggressive plaque pathology, we wanted to determine whether BACE1 gene deletion likewise would prevent plaque formation in these animals. A qualitative assessment of the presence or absence of plaques in 13-month-old PDAPP/BACE(+/+) and PDAPP/BACE(−/−) mice showed a complete absence of amyloid plaques in the mice with the latter genotype (Fig. 2, A1 and A2). The plaque burden observed in the PDAPP/BACE(+/+)
mice was typical for PDAPP (109) animals at 13 months of age. At 13 months, the vast majority of Aβ in brains of PDAPP mice is deposited in amyloid plaques, and therefore, ELISA measurements of Aβ in guanidine-solubilized cortex provides another measurement of amyloid deposition (25). To verify that immunohistochemical analysis did not miss some forms of amyloid deposits, total Aβ load was quantitated by ELISA in solubilized cortex, which also showed a complete lack of Aβ in PDAPP/BACE(−/−) mice (Fig. 1B). Thus, the production of Aβ and its deposition into plaques can be completely ablated by BACE1 gene knock-out in PDAPP mice.

Dystrophic neurites co-localize with senile plaques in transgenic hAPP mouse models (15, 31) and in humans (32–34), and several lines of evidence in vitro (35) and in vivo (36) indicate that they form in response to the appearance of plaques. These pathologic features consist of swollen and distorted neurites, which can be visualized with APP-specific antibodies because they accumulate a variety of proteins including APP. The same animals investigated for amyloid plaques were also assessed for neuritic dystrophy (Figs. 2, B1 and B2). All of the PDAPP/BACE(−/−) mice were devoid of dystrophic neurites, thus showing that BACE1 gene knock-out can also prevent plaque-dependent AD-like pathology in PDAPP mice.

Heterozygous BACE1 Gene Knock-out Has a Modest Effect on Soluble Brain Aβ Levels in Young Mice Yet Dramatic Effects on the Development of Plaques with Age—Complete deletion of the BACE1 gene leads to subtle behavioral and electrophysiological alterations in normal and in APP transgenic mice (11, 37). In aged mice lacking BACE1, we observed abnormally elevated synaptophysin levels in the frontal cortex and hippocampal molecular layer (see below). These observations support the notions that BACE1 may have physiological targets other than

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FIGURE 1. Homozygous BACE1 gene deletion completely eliminates Aβ in cortex of young and aged PDAPP mice. Guanidine-solubilized cortices from 3- (A) or 13-month-old (B) PDAPP/BACE(+/+ ) (PDAPP mice with BACE1 gene) and PDAPP/BACE(−/−) (PDAPP mice with both alleles of the BACE1 gene deleted) mice were analyzed for total Aβ by ELISA as described under “Experimental Procedures.” All samples from PDAPP/BACE(−/−) were below the level of detection.

FIGURE 2. Amyloid plaques and dystrophic neurites do not develop in PDAPP/BACE−/− mice and are greatly reduced in PDAPP/BACE+/− mice. Thirteen-month-old mice each from each genotype (A1 and B1, PDAPP/BACE(+/+ ); A2 and B2, PDAPP/BACE(−/−); A3 and B3, PDAPP/BACE(+−/−), n = 4–12/group) were subjected to histopathological analyses of plaques (A) and of dystrophic neurites (B) as described under “Experimental Procedures.” Panels A1–A3 and B1–B3 show representative images of the median for each group of animals. Amyloid plaques are apparent in both cortex and hippocampus, whereas dystrophic neurites at this age are most prominent in the hippocampus. Dystrophic neurites in PDAPP/BACE(+/−) mice are indicated by an arrow. Note the absence of plaques and of dystrophic neurites in PDAPP/BACE(−/−) mice and the greatly reduced plaque and neuritic burden in PDAPP/BACE(+/−) mice compared with PDAPP/BACE(+/+) mice. Scale bars, 500 μm (main panels), 120 μm (insets).
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APP (38–44) and/or that APP fragments altered by BACE1, such as the Aβ peptide, may have some essential physiological role (45). Thus, complete inhibition of BACE1, especially over long periods of time, may be linked to mechanism-based toxicities, and therefore, partial inhibition of BACE1 may be a more viable therapeutic approach. To evaluate the effect of partial BACE1 inhibition on AD-like pathologies in PDAPP mice, we generated PDAPP mice in which only one of the BACE1 alleles was deleted (PDAPP/BACE(+/-)) mice (see “Experimental Procedures”). BACE1 activity in the brain of these mice was reduced by 50% (Ref. 7 and Fig. 3F), in concordance with the reduction in gene copy number.

We analyzed Aβ load in the brain of 3-, 13-, and 18-month-old PDAPP/BACE(+/-) mice, comparing them to PDAPP mice with the full set of two BACE1 alleles (PDAPP/BACE(+/+) mice). Surprisingly, we found that the 50% reduction of BACE1 activity in PDAPP/BACE(+/-) mice led only to a small (∼12%), albeit significant, reduction in total transgene-derived Aβ and Aβ-42 levels in the brains of three-month-old animals (Figs. 3, A and F) and to a similarly small reduction of transgene-derived total Aβ in blood of 3- and 13-month-old PDAPP/BACE(+/-) mice (see supplemental Fig. 1). This reduction was reproducible and robust and was documented in the brains of three large groups of 2–4-month-old animals bred in different generations (n = 37–47 for each genotype in each group, data not shown). We also observed a comparable reduction in Aβ levels in whole brains from 7-day-old animals (data not shown), further confirming that there is a reproducible effect on the steady-state Aβ levels in brains of young animals. We have previously reported that the ratio of Aβ-42 to total Aβ is a critical determinant for the development of plaques in APP transgenic mice (23), and this has been confirmed by results showing that Aβ-40 inhibits amyloid deposition (46). We, therefore, measured the ratio of Aβ-42 to total Aβ and found that there was no difference between PDAPP/BACE(+/+) and PDAPP/BACE(+/-) animals (data not shown).

FIGURE 3. Heterozygous BACE1 gene deletion causes minimal reduction of Aβ levels in young mice but dramatic reduction of Aβ levels in the brains of older PDAPP mice. Aβ levels were measured by ELISA analyses of guanidine-solubilized brain extracts as described under “Experimental Procedures.” Panels A1 and A2 show neocortical total Aβ and Aβ-42 levels, respectively, of 3-month-old PDAPP/BACE(+/+) and PDAPP/BACE(+/-) mice. The difference in Aβ levels between these groups at this age was minimal (∼12%). Because at 3 months of age, no plaques were present (not shown), the Aβ measurements reflect the pool of soluble Aβ species. Panels B1 and B2 show neocortical and hippocampal total Aβ levels of 13-month-old, and panel C shows neocortical Aβ levels of 18-month-old PDAPP/BACE(+/+) and PDAPP/BACE(+/-) mice. At these ages plaques could be detected in the brains of mice of both genotypes (see Fig. 2), and therefore, Aβ levels reflect total Aβ load. Differences in Aβ load between mice of these two genotypes were ∼90% at 13 months and 50% at 18 months. n.s., not significant. Panel D depicts the age-dependent progression of neocortical total Aβ in PDAPP/BACE(+/-) and PDAPP/BACE(+/+) mice from the same cohort of animals analyzed in panels A–C. Panel E shows the cortical β-sAPP levels in these mice measured by ELISA analyses of guanidine-solubilized brain extracts as described under “Experimental Procedures.” There were 40–43 animals per group, and p values were determined by the Mann-Whitney test. The relative amounts of β-sAPP in these two groups does not change with age. Panel F shows the β-secretase enzyme activity in crude membrane fractions from hemibrains of wild type and heterozygous and homzygous BACE knock-out animals (n = 4 each). β-Secretase enzyme activity corresponds to BACE gene copy number.

We have developed an approach to partially inhibit BACE1 activity, thereby delaying the development of plaques in mice, and this approach may be relevant for AD therapy.
the ongoing processing both preceding and during plaque formation.

In heterozygous PDAPP mice, plaque formation starts sometime between 5 and 7 months and continues to increase as the mice age (25). We, therefore, asked whether the small decrease in Aβ levels seen in the brain of 3-month-old PDAPP/BACE(+/−) mice led to an observable effect on plaque formation in older mice. Given the animal to animal variability of transgene-derived Aβ in the brain of PDAPP mice, there was a significant overlap in brain total Aβ and Aβ-42 levels of 3-month-old PDAPP/BACE(+/−) and PDAPP/BACE(+/+) mice (Fig. 3, A1 and A2), and thus, we did not expect to see appreciable differences in the formation of plaques at later ages between these two genotypes. Quite stunningly, however, we found that brain Aβ load of PDAPP/BACE(+/−) mice was very substantially reduced (~90% at 13 months (Fig. 3, B1 and B2); ~50% at 18 months (Figs. 3, C and D)) compared with that of PDAPP/BACE(+/+) mice. This result shows that a minimal decrease of soluble Aβ in young PDAPP mice has a dramatic effect on plaque formation at later ages and suggests both that the relationship of Aβ levels to plaque level is a non-linear phenomenon, as has been suggested by biophysical measurements (47), and also that Aβ levels alone do not drive the development of amyloid plaque.

To confirm that the ELISA measurements of Aβ in older PDAPP mice did indeed reflect plaque pathology, we visualized Aβ plaques on sections of 13-month-old PDAPP/BACE(+/−) and PDAPP/BACE(+/+) mice by immunostaining with an anti-Aβ antibody (3D6) and analyzed the results by quantitative image analysis. The general appearance of the plaques was similar for both genotypes (Fig. 2A3). However, the plaque burden in PDAPP/BACE(+/−) mice was reduced 5-fold compared with that in control (Fig. 4A). This correlates well with the quantitation of plaques using the Aβ ELISA and confirms the conclusion that a very small reduction in Aβ levels in brain in young mice leads to a dramatic reduction in the development of plaques in older animals.

We next wanted to determine whether dystrophic neurites, which surround and invade amyloid plaques, were altered in the PDAPP/BACE(+/−) mice in a manner corresponding to the reduction in amyloid plaques. We found that the amount of dystrophic neurites in 13-month-old PDAPP/BACE(+/−) mice was reduced 4-fold compared with age-matched PDAPP/BACE(+/+) mice, reflecting the reduction in plaques in these mice (Figs. 2B3 and 4B). The distribution and size of the dystrophic neurites was the same in mice of both genotypes, indicating that there was no major alteration in the nature of the dystrophic neurites (data not shown). Thus, plaque-associated neuritic dystrophy, an age-dependent neurodegenerative pathology of PDAPP mice, is also ameliorated by a modest reduction of Aβ levels at young ages.

Elimination of Cerebral Aβ Blocks the Development of Synaptophysin Loss in PDAPP Mice—Loss of synapses and of the associated presynaptic protein synaptophysin (SYN) is a key pathological feature of AD that correlates robustly with cognitive deficits (13, 14, 48). Indirect evidence supports the notion that soluble Aβ assemblies and not Aβ plaques are responsible for synaptic toxicity in APP transgenic mice (23, 49–52). SYN deficits correlate with levels of soluble Aβ but do not correlate with plaque load on an animal by animal basis (23, 50). Therefore, quantitation of SYN provides a measure of plaque-independent AD-like pathology in the brains of PDAPP mice. To directly test the link between Aβ and synaptic deficits, we measured SYN in the frontal cortex and the hippocampal OML of PDAPP mice and of wild type controls both with and without the BACE1 gene at 3, 10–13, and 18 months of age (Fig. 5). The SYN deficit in the hippocampal OML and in the frontal cortex of 10–13-month-old PDAPP/BACE(+/+) mice was absent in PDAPP/BACE(−/−) mice. This indicates that BACE1 deletion and, thus, the absence of Aβ prevents synaptic pathology in PDAPP mice at these ages. These data also suggest that the synaptic deficit seen at 3 months of age, before the onset of plaques, may also be ameliorated by BACE1 deficit, although this trend did not reach statistical significance. The validity of this trend is supported by the observation that in a similar line of hAPP transgenic mice the SYN deficit at 3 months correlates with soluble Aβ (23). Our study shows that by eliminating Aβ in PDAPP mice containing the hAPP transgene, the hippocampal SYN deficit is prevented, thus further establishing the role of soluble Aβ species in AD-related synaptic pathology.

No SYN deficit was detected in 3- and 10–13-month-old WT/BACE(−/−) mice and PDAPP/BACE(−/−) mice. However, an increase in SYN in both of the analyzed brain regions was detected in these mice at 18 months. When the BACE knock-out is combined with homozygous PDAPP mice by 6 months of age, PDAPP/BACE(−/−) bigenic animals showed an increase in SYN, whereas no increase was seen in WT/BACE(−/−) animals at that age (supplemental Fig. 2). This result indicates that BACE1 deletion has a synaptotrophic effect in PDAPP mice, which is accelerated by the presence of two copies of the hAPP transgene. The absence of BACE1 may shift the processing of endogenous and transgenic APP toward the generation of trophic moieties that exert their effect as a function of both APP expression levels and time. Consistent with this interpretation, APP products have been shown to provide neuroprotective functions in transgenic animals (53), and α-sAPP has been shown to be increased in BACE(−/−) mice (9).
of the BACE1 and Aβ reductions required for significant efficacy. Using BACE1 knock-out mice, we have completely ablated Aβ production in the PDAPP transgenic mice and shown that development of both plaque-related amyloid deposition and neuritic dystrophy and plaque-independent synaptic deficits are blocked in PDAPP/BACE1(−/−) animals. This directly demonstrates Aβ-mediated neurotoxicity in vivo and also demonstrates that eliminating BACE1 can reverse this pathology. We further show that although 50% reduction in BACE1 results in only small decreases in the levels of total Aβ and the aggregation prone Aβ42, the impact on the development of both plaque and synaptic pathologies in PDAPP animals is dramatic. Thus, to be therapeutically effective, it may only be necessary to inhibit a fraction of the endogenous BACE1 activity resulting in very small decreases in Aβ levels. Partial inhibition of BACE1 in a therapeutic setting is more desirable as it obviates potential deleterious effects.

The loss of synapses is a cardinal feature of AD. Numerous studies report that synaptic pathology is a much better correlate of cognitive deficits in AD than densities of Aβ plaques and tangles or neuronal death (13, 14, 48). Multiple indirect lines of evidence support the notion that soluble, oligomeric Aβ species, and not Aβ plaques, are responsible for synaptic toxicity in hAPP mice (23, 49–52) and, at least in part, in humans (54, 55). Therefore, in addition to showing that plaque pathology is eliminated by the absence of BACE1, as shown previously (10), we have also demonstrated that a plaque-independent neuropathology, the loss of the presynaptic protein synaptophysin, is reversed in the BACE1(−/−) animals. Importantly, even partial reduction of BACE1 in the heterozygous knock-out dramatically delayed the progressive age-dependent synaptic deficit seen in PDAPP mice.

These studies demonstrate that even partial BACE1 inhibition has the potential to protect against synapto-toxicity as well as plaque pathology. The alleviation of neurodegenerative pathologies in PDAPP/BACE1(−/−) bigenic animals indicates that these pathological features are driven by Aβ toxicity and not by ectopic expression of the APP transgene. There remains the possibility that BACE1 cleavage of APP could indirectly affect APP products other than Aβ. For example, the C-terminal 100-amino acid fragment of APP, a direct product of BACE1, has been shown to be neurotoxic (56). However, most likely Aβ is the offending molecule. Given that synaptophysin

**FIGURE 5.** Homozygous BACE1 gene deletion abrogates SYN deficits in both young and aged PDAPP mice. In A, the upper panel shows quantitation of SYN levels in the frontal cortex, and the lower panel shows quantitation of SYN levels in the hippocampal OML of PDAPP mice and wild type controls, both with or without the BACE1 gene. In B, panels show images of SYN-positive presynaptic terminals of the frontal cortex from 10–13- and from 18-month-old WT or PDAPP/BACE1(−/−) mice. The SYN levels of 3- and of 10–13-month-old WT/BACE1(+/−) mice are increased compared with WT/BACE1(+/+) mice, indicating a synaptotrophic effect. Results shown in A are the means ± S.E. There were 9–47 animals per group. p < 0.05 (*), p < 0.01 (**), and p < 0.001 (***), values were determined by Dunnett’s test compared with WT/BACE1(+/+) controls. Scale bar, 50 μm.

**Heterozygous Knock-out of BACE1 Gene Delays Age-related Synaptic Deficits in PDAPP Mice—**To investigate the effect of deletion of one BACE1 allele on AD-related, plaque-independent synaptic pathology, we analyzed SYN in the frontal cortex and hippocampal OML in PDAPP/BACE1(+/−) mice and compared them to WT/BACE1(+/+) and PDAPP/BACE1(+/+) mice (Fig. 6). In contrast to PDAPP/BACE1(+/+) mice, 3 and 10–13-month-old PDAPP/BACE1(−/−) mice showed no significant SYN deficits compared with WT/BACE1(+/+) mice in the frontal cortex and hippocampal OML. Eighteen-month-old PDAPP/BACE1(−/−) mice showed SYN deficits in these two brain regions. However, their SYN deficits were significantly less severe in the neocortex (with a similar trend in the hippocampal OML) than those found in age-matched PDAPP/BACE1(+/+) mice and were comparable with those found in 10–13-month-old PDAPP/BACE1(+/+) mice. These results indicate that a modest lowering of Aβ levels through BACE1 inhibition can delay SYN deficits in PDAPP mice.

**DISCUSSION**

As an attractive target for AD therapeutic intervention, it is critical to ascertain both the full spectrum of pathologies anticipated to be alleviated by BACE1 inhibition and the magnitude
Heterozygous BACE1 gene deletion delays SYN deficits in PDAPP mice.

In A, the upper panel shows quantitation of SYN levels in the frontal cortex, and the lower panel shows quantitation of SYN levels in the hippocampal (OML) of PDAPP mice either with or without partial BACE1 gene deletion and of wild type controls. In hippocampal (OML) of PDAPP mice either with or without partial BACE1 gene deletion and of wild type controls. In frontal cortex, and the lower panel shows quantitation of SYN levels in the hippocampal (OML) of PDAPP mice either with or without partial BACE1 gene deletion of wild type controls. In frontal cortex, and the lower panel shows quantitation of SYN levels in the hippocampal (OML) of PDAPP mice either with or without partial BACE1 gene deletion of wild type controls.

Partial BACE1 Reduction Inhibits Alzheimer Pathology

PDAPP mice.

FIGURE 6. Partial BACE1 Reduction Inhibits Alzheimer Pathology

Deficits correlate with Aβ and not APP levels in multiple transgenic mouse lines (23) and can be specifically modified by passive immunization with anti-Aβ antibodies that have no effect on APP (50), the parsimonious conclusion is that Aβ itself is causing synaptic deficits in the PDAPP model.

Recent reports have shown some deficits in behavior and partial lethality in BACE1(−/−) mice (11, 12). Although these data suggest potential concerns for total inhibition of BACE1 in a therapeutic setting, these phenotypes are not seen in the heterozygous BACE1(+−/−) animals. The significant reduction of pathology in PDAPP/BACE(+−/−) animals reported here indicates that partial inhibition of BACE1 would be expected to be therapeutically efficacious without having negative consequence.

The exquisite sensitivity of plaque formation to small changes in Aβ concentration is consistent with an extended latency period for oligomer formation and fibril initiation that is dependent on multiple species of Aβ coming together. This model predicts a non-linear relationship between development of plaques and Aβ levels in the brain. 2-Fold changes in APP transgene levels in homozygous PDAPP mice and in lines expressing different levels of APP also lead to significant changes in the development of plaques (Ref. 23 and data not shown). Thus, at least part of the exaggerated response of plaque formation to Aβ levels is consistent with models of increased latency due to a requirement for multiple Aβ molecules to assemble.

A model invoking a non-linear relationship between Aβ levels and plaque burden explains how a relatively small change in median levels of Aβ can drive dramatic differences in the median levels of amyloid burden. However, the large degree of animal to animal variability and consequential high degree of overlap in the distribution of Aβ brain levels in young PDAPP/BACE(+−/−) versus PDAPP/BACE(+−/−) mice have implications not fully explained by this type of model. Assessment of the distribution of Aβ brain levels in PDAPP/BACE(+−/−) versus PDAPP/BACE(+−/−) mice indicates that the effect of a partial reduction in BACE1 on Aβ levels in young mice is not the sole determinant of the development of Aβ plaque pathology. An important feature of this study was that very large numbers of animals were used. As a consequence, we could clearly determine the degree of overlap in the distributions of brain Aβ levels for these two genotypes in young animals and compare that to the degree of overlap in the distributions of Aβ amyloid deposition in older animals. Although young animals showed a significant degree of overlap in total Aβ and Aβ-42, at 12 months of age these same two groups showed significant divergence and much less overlap in plaque deposition. This indicates that young PDAPP/BACE(+−/−) animals with overall levels of Aβ equivalent to those in some PDAPP/BACE(+−/−) mice have a different outcome with respect to the development of plaques with age. The ratio of Aβ-42 to total, an important determinant of plaque formation (23, 46), did not differ between PDAPP/BACE(+−/−) and PDAPP/BACE(−−/−) animals. Thus, neither changes in overall total Aβ nor Aβ-42 levels nor the ratio of the two is the sole determinant in the reduction of plaque pathology mediated by BACE1 reduction.

One possible explanation for these data is that effects of BACE1 on other substrates (38–43, 57) play a role in determin-
ing plaque deposition. A more parsimonious explanation is that a subfraction of Aβ, perhaps at particular cellular locations, is critical for the seeding and progression of plaques. To explore this we have measured Aβ in brain fractions but as yet have not identified a critical compartment. If the effects on plaque development are driven by Aβ alone, one would predict that low levels of γ-secretase inhibition, demonstrating small effects on reduction of Aβ levels, could also have a dramatic effect on plaque and synaptic pathologies. Such a finding could significantly impact the amounts of drug ultimately required for therapeutic benefit for both BACE1 and γ-secretase inhibitors.

While this manuscript was in preparation Laird et al. (11) also reported a delay in plaque formation in BACE(+/-) mice. However, the quantitative relationship between Aβ formation in young animals and the dramatic differences in the development of plaques that we present was not determined in that study. This could have been the result of using fewer animals or been due to the difficulty in distinguishing Aβ levels in brains of animals ranging from 7 days to 4 months of age and have not seen any time-dependent changes in Aβ for either the PDAPP/BACE(+/-) control or the PDAPP/BACE(+/-) animals during this time. Furthermore, the relative amounts of Aβ of these two groups are also reflected in blood levels for both young and aged animals. We are, thus, confident that our measurements of steady-state Aβ levels in young animals truly reflect the relative amounts of Aβ throughout the extended period before the onset of plaque formation. Another difference between the two studies is that Laird et al. (11) used an APP transgenic animal containing the Swedish familial AD mutation, which is more efficiently cleaved by BACE1 than is the wild type APP (30, 58). This combined with the presenillin transgene results in an aggressive model of plaque development, which may have obscured the dramatic effects of the heterozygous BACE knockout on plaque development that we were able to demonstrate at 12 months in the PDAPP model.

In summary, complete or partial inhibition of BACE1 can profoundly ameliorate both the plaque-related and the plaque-independent pathologies in an APP transgenic mouse model of Alzheimer disease. The heterozygous BACE1 knockout mice displayed significant delays in the onset of plaque and synaptic pathologies without showing any evidence of toxicity. These results suggest the possibility that very small reductions in Aβ levels might result in long-term significant reduction in plaque burden and synaptic deficits in patients suffering from AD, which might result in significant therapeutic benefit.

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