EMBO Molecular Medicine

β- but not γ-secretase proteolysis of APP causes synaptic and memory deficits in a mouse model of dementia

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Keywords: Alzheimer disease; BACE1; BRI2; familial Danish dementia; mouse models

DOI 10.1002/emmm.201100195

Received September 09, 2011
Revised November 30, 2011
Accepted December 05, 2011

A mutation in the *BRI2/ITM2b* gene causes loss of BRI2 protein leading to familial Danish dementia (FDD). BRI2 deficiency of FDD provokes an increase in amyloid-β precursor protein (APP) processing since BRI2 is an inhibitor of APP proteolysis, and APP mediates the synaptic/memory deficits in FDD. APP processing is linked to Alzheimer disease (AD) pathogenesis, which is consistent with a common mechanism involving toxic APP metabolites in both dementias. We show that inhibition of APP cleavage by β-secretase rescues synaptic/memory deficits in a mouse model of FDD. β-cleavage of APP yields amino-terminal-soluble APPβ (sAPPβ) and β-carboxyl-terminal fragments (β-CTF). Processing of β-CTF by γ-secretase releases amyloid-β (Aβ), which is assumed to cause AD. However, inhibition of γ-secretase did not ameliorate synaptic/memory deficits of FDD mice. These results suggest that sAPPβ and/or β-CTF, rather than Aβ, are the toxic species causing dementia, and indicate that reducing β-cleavage of APP is an appropriate therapeutic approach to treating human dementias. Our data and the failures of anti-Aβ therapies in humans advise against targeting γ-secretase cleavage of APP and/or Aβ.

INTRODUCTION

Amyloid deposition of amyloid-β (Aβ) peptide characterises Alzheimer disease (AD). Aβ derives from sequential cleavage of amyloid-β precursor protein (APP) by β- and γ-secretases (Cole & Vassar, 2007; De Strooper et al, 2010; Fig 1A). Interestingly, mutations in either *APP* or the γ-secretase genes *PSEN1* and *PSEN2* cause familial AD (FAD; Bertram et al, 2010; St George-Hyslop & Petit, 2005). Mutation of *BRI2/ITM2b* causes familial Danish dementia (FDD), an AD-like familial dementia with amyloid deposits. In normal individuals the immature BRI2 precursor (imBRI2) is cleaved by convertases in the Golgi into mature BRI2 (mBRI2) and a carboxy-terminal 23 amino acid peptide (Bri23). mBRI2 is transported to the plasma membrane and Bri23 is secreted. In the Danish kindred, the presence of a 10-nt duplication one codon before the normal stop codon produces a frame-shift in the BRI2 sequence generating a larger-than-normal precursor protein called BRI2ADan. Cleavage by convertases releases the amyloid subunit that comprises the last 34 COOH-terminal amino acids (ADan) and mBRI2. ADan accumulates into amyloid plaques, which contain both Aβ and ADan (Choi et al, 2004; Vidal et al, 2000).

Since amyloidogenic peptides are believed to cause dementias (Hardy & Selkoe, 2002), transgenic mice carrying mutant *APP*, *PSEN1/2* or *BRI2/ITM2b* are used to model these dementias, as over-expression is necessary to reproduce amyloidosis (Jucker, 2010). However, over-expression of mutant genes might produce harmful effects unrelated to dementias and lead to erroneous information concerning the pathogenesis and therapy of human diseases. The clinical

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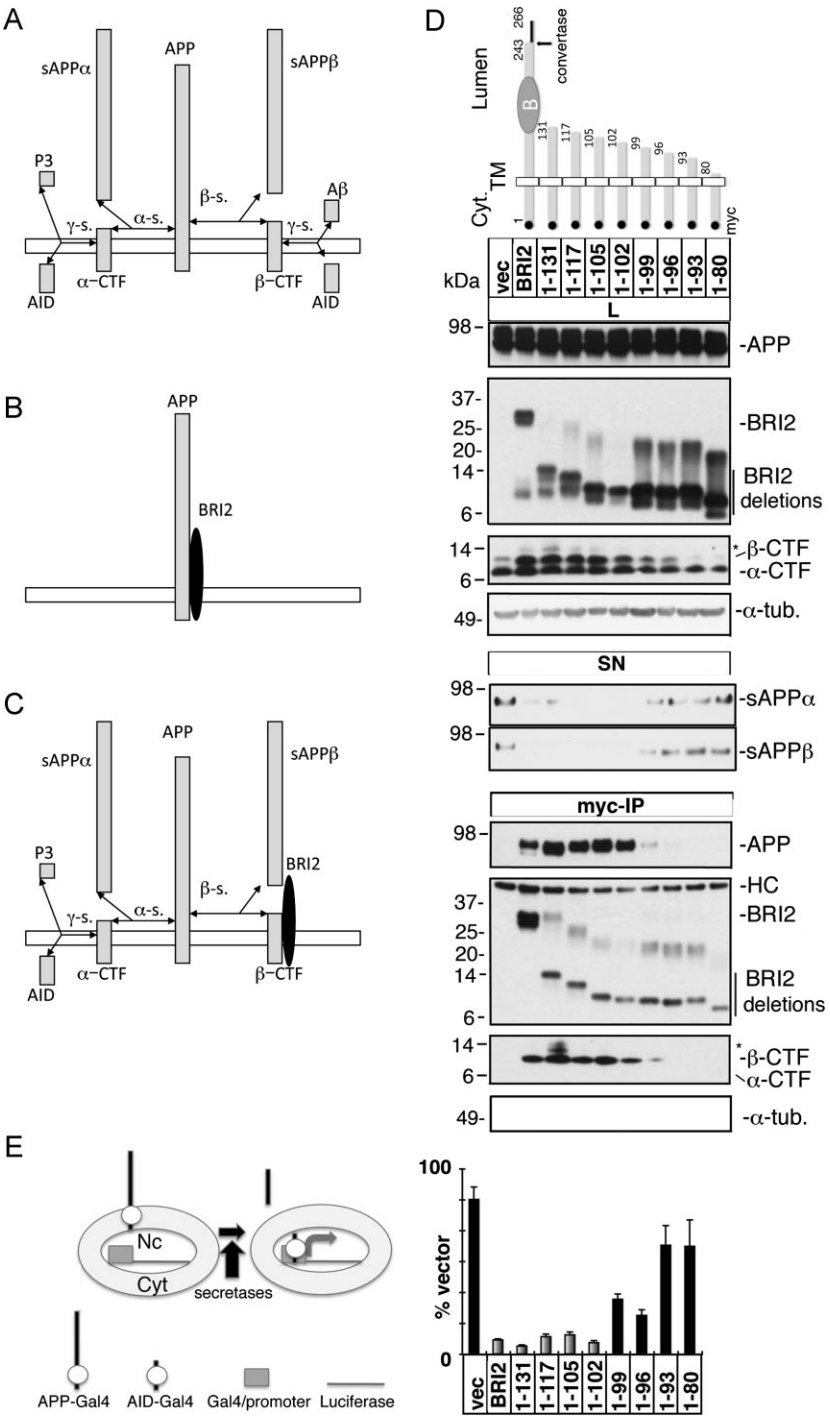

Figure 1. Mapping the BRI2 domain that binds APP and inhibits APP processing.

A. APP is cleaved by β-secretase into sAPPβ and β-CTF. γ-cleavage of β-CTF yields Aβ and AID/AICD peptides. Alternatively, α-secretase clips APP into sAPPα and α-CTF. α-CTF is cut by γ-secretase into P3 and AID.

B-C. BRI2 binds APP and inhibits processing by α- and β-secretases. Binding of BRI2 to β-CTF inhibits cleavage by γ-secretase.

D. Constructs and domains [cytoplasmic (Cyt), transmembrane (TM), extracellular (Lumen), brichos (B) and convertases-cleavage site, myc-tag]. Lysates (L) and α-myc immunoprecipitates (myc-IP) from transfected cells were analysed by Western blot (WB) for α-Tubulin, BRI2, APP and APP-CTFs. Supernatants (SN) were analysed for sAPPα and sAPPβ. *Indicates an APP-CTF larger than β-CTF, which is routinely observed when BRI2 is over-expressed. This band, whose origin is unknown, also binds to BRI2.

E. APP-Gal4, AID-Gal4, Gal4-depended promoter, luciferase reporter, cytoplasm (Cyt) and nucleus (Nc) are schematically indicated. Luciferase activity is expressed as % of the activity in cells transfected with APP-Gal4, luciferase reporter and empty vector (vec). Overall, our analysis shows that BRI2 residues comprised between amino acids 102 and 134 retained APP-binding properties and inhibitory effects on APP processing.

failures of compounds efficacious in transgenic models support this hypothesis (Ganjei, 2010). To avoid artefacts of over-expression, we generated a knock-in mouse model of FDD (FDD_{KI}) that, like FDD patients (Vidal et al, 2000), is heterozygous for one mutated FDD allele of *BRI2/ITM2b* (Giliberto et al, 2009). FDD_{KI} mice develop progressive synaptic and memory deficits due to loss of mBri2, with no amyloidosis (Tamayev et al, 2010b). mBRI2 binds mature APP and inhibits APP processing (Fotinopoulou et al, 2005; Matsuda et al, 2005; Matsuda et al, 2008; Matsuda et al, 2011a; Fig 1B and C); owing to the loss of mBRI2, APP processing is increased in FDD (Matsuda et al, 2011b; Tamayev et al, 2011). Remarkably, memory and synaptic deficits of FDD_{KI} mice require APP (Tamayev et al, 2011), providing genetic evidence that APP and BRI2 functionally interact, and that APP mediates FDD neuropathology.

RESULTS

The BRI2 domain that binds APP and inhibits APP processing maps to amino acids 74–102

To test if the loss of mBRI2 in FDD impairs memory via toxic APP metabolites resulting from processing, we searched for BRI2-derived peptides that replicate the inhibitory function of BRI2 on APP-cleavage. mBRI2 interacts with mature APP and β-carboxyl-terminal fragments (β-CTF), and increases the levels of β-CTF by inhibiting its γ-cleavage (Matsuda et al, 2005; Matsuda et al, 2008; Fig 1B and C). The inhibitory domain was previously mapped to the extracellular region of BRI2 (amino acids 74–131; Matsuda et al, 2005). To define it further, HeLa cells were co-transfected with APP (HeLa-APP) and myc-tagged BRI2 fragments progressively deleted from the COOH-terminus (Fig 1D). APP and BRI2 constructs were expressed at similar levels (Fig 1D). Binding to APP/β-CTF and β-CTF accumulation were progressively abolished particularly between positions 102 and 93 (Fig 1D). The levels of α-Tubulin were similar in all transfected cells and α-Tubulin was not precipitated by anti-myc, further underlying the specificity of the mBRI2/mAPP interaction. In addition, the BRI2 deletion constructs that bind APP also reduced the levels of both sAPPβ and sAPPα (Fig 1D). To corroborate these effects on APP processing, BRI2 constructs were co-expressed with an APP-Gal4 fusion construct and a luciferase-reporter under the control of a Gal4-dependent promoter. APP-Gal4 is a fusion of the yeast transcription factor Gal4 to the cytoplasmic domain of APP. Cleavage of APP-Gal4 releases the APP intracellular domain (AID)-Gal4 fusion-protein that drives luciferase expression (Gianni et al, 2003; Fig 1E). BRI2 blocked most AID-Gal4 release-dependent luciferase activation, but C-terminal deletion again from position 102 to 93 progressively lost this inhibitory activity (Fig 1E). Thus, the functional domain of BRI2 mapped from amino acids 74 to 102.

A BRI2-derived peptide binds APP and inhibits β-cleavage of APP

We tested if peptides spanning this domain duplicated BRI2's function. Two overlapping peptides N3 and N8 strongly reduced β-cleavage and moderately decreased α-processing of APP (Fig 2A). Mutagenesis of N3 showed that replacing any of amino acids 3–10 with Alanine reduced the inhibitory activity of N3 on β-cleavage of APP, showing the functional importance of these residues. However, replacing either the first or second residue (N3-1A/N3-2A) actually resulted in a stronger inhibitor of APP processing by β-secretase (Fig 2B). Notably, α-cleavage of APP is inhibited by N3, unaffected by N3-2A and, probably, increased by N3-1A. It is possible that these three peptides bind APP differently thereby reducing (N3), unaffecting (N3-2A) or increasing (N3-1A) access of α-secretase to the APP-docking/cleavage site. However, the mechanism underlying this potentially useful difference remains to be investigated.

Since N3-2A has a strong and specific inhibitory activity on β-cleavage of APP, we further analyse the effects of N3-2A on APP processing. We measured the levels of APP-CTFs in HEK293-APP cells treated with N3-2A, control peptide N1 or media alone. APP-CTFs are rapidly turned over in cells by γ-secretase. Thus, to reduce the confounding effects of APP-CTFs' turnover, we performed a parallel experiment in which cells were treated with the γ-secretase inhibitor (GSI) compound-E. As shown in Fig 2C, N3-2A reduces the levels of β-CTF as compared to cells treated with either media alone or N1, which is consistent with the inhibition of β-cleavage of APP. The α-secretase-derived APP-CTF, α-CTF, is not altered by N3-2A, again consistent with the fact that N3-2A does not change the levels of sAPPα (Fig 2C). Similar data where obtained in HeLa-APP cells (Fig 2C).

To better characterize the mechanism of action of N3-2A, we tested whether this peptide binds APP. To this end, HeLa-APP cells were cultured with or without N3-2A fused to a C-terminal Flag epitope (N3-2A-F). After 2 h of incubation, cell lysates were precipitated with α-Flag-agarose-beads and co-precipitated molecules were eluted with a Flag-peptide. Like BRI2, N3-2A-F binds mature APP (Fig 2D). Specificity of this interaction was confirmed by showing that untagged N3-2A could compete for binding to APP (Fig 2E), and that peptides that do not inhibit APP processing (N4-F or the single amino acid N3 mutant N3-4A-F, Fig 2A and B), did not bind APP (Fig 2F). To determine whether these biological properties of N3-2A, described in cancer cell lines over-expressing APP, are conserved when using brain cells expressing endogenous APP, we tested if N3-2A-F binds endogenous APP from freshly isolated murine brain cells. As shown in Fig 2G, N3-2A-F/endogenous APP complexes are readily detectable.

N3-2A-F/APP complexes were detected only in metabolically active cells (Fig 2D). To determine how cell metabolism influences formation of N3-2A/APP complexes, HeLa-APP cells were surface-biotinylated, and cultured with N3-2A-F. After incubation, half of the cells were treated with a reducing reagent, which removes biotin from plasma membrane but not from internalised proteins (+red). N3-2A-F/APP complexes were isolated and further precipitated with streptavidin-beads. In the non-reduced (-red) sample most of APP bound to N3-2A-F was biotinylated (Fig 2H), suggesting that N3-2A-F binds APP on the cell surface. In the reduced sample, N3-2A-F/APP complexes were found both in intracellular compartments (biotinylated APP) and on the plasma membrane (non-biotinylated APP) (Fig 2H), supporting the hypothesis that part of plasma membrane N3-2A-F/APP complexes are internalised.

BRI2 binds the region of APP comprising the β-cleavage site, thereby blocking access of this protease to APP, while β-secretase is still active on other substrates (Fotinopoulou et al, 2005). N3-2A did not inhibit the activity of purified β-secretase, while the well-characterized β-secretase-inhibitor IV did (Fig 2I), indicating that N3-2A has a mechanism of action similar to BRI2 and blocks β-cleavage of APP but not β-secretase. Thus, we will refer to N3-2A as a modulator of β-cleavage of APP (MoBA). It should be noted that unlike full-length BRI2, MoBA does not bind β-CTF (Fig 2D and E) and does not overtly inhibit α-processing of APP (Fig 2B). These data suggest that N3-2A/MoBA interferes predominantly with processing of APP by β-secretase and does not modulate γ-cleavage of β-CTF (Fig 2J).

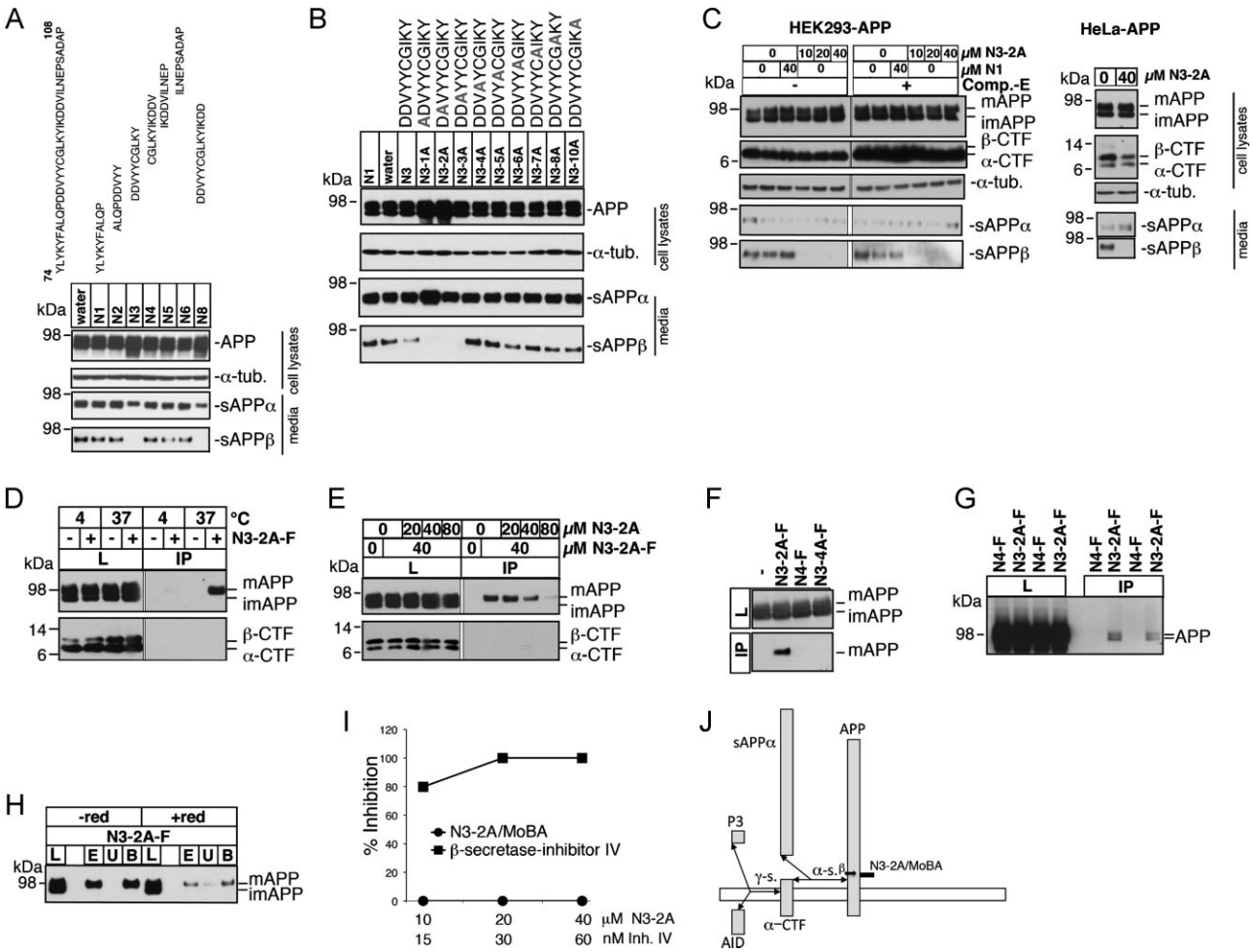

Figure 2. A BRI2-derived peptide binds APP and inhibits β-cleavage of APP.

A-B. HEK293-APP cells were incubated with the indicated peptides. β- and α-cleavage of APP were quantified by measuring sAPPβ and sAPPα in media by WB. WB of cell lysates detected APP and α-Tubulin.

C. WB analysis of cell lysates and conditioned media from HEK293-APP cells treated either with the indicated concentrations of either N3-2A or N1. In a duplicate experiment, cells were treated with compound-E (+) while incubated with the indicated peptides. Lysates were probed for APP, APP-CTFs and α-Tubulin, culture media was probed for sAPPα and sAPPβ. In the right panel, the results of a similar experiment in HeLa-APP cells are shown.

D-F. WB analysis of lysates (L) or α-Flag IP (IP) from HeLa/APP cells incubated for 2 h with Flag-tagged peptides.

D. Cells were incubated at either 37 or 4 °C with or without 40 μM N3-2A-F.

E. The indicated concentrations of N3-2A were added to the media containing 40 μM N3-2A-F.

F. Cells were incubated with 40 μM N3-2A-F, N4-F or N3-4A-F.

G. Brain cells were cultured as in (**D**).

H. Biotinylated cells were cultured as in (**D**). The reduced and not reduced samples are indicated (+red and −red, respectively). Lysates (L), α-Flag IP eluted with Flag-peptide (E), eluted sample precipitated with streptavidin-beads [both the fraction unbound (U) and bound (B) to streptavidin-beads], were probed for APP in WB.

I. Purified β-secretase was incubated with fluorescent β-secretase substrate for 30 min, resulting in β-cleavage that could be detected by fluorescence increase. In separate samples, the indicated concentrations of N3-2A or β-secretase-inhibitor IV were added to the reaction. The data are shown as % of inhibition of β-secretase activity in samples without inhibitors.

J. Model depicting the mechanism of action of N3-2A/MoBA. The peptide interferes with processing of APP by β-secretase but, unlike full-length BRI2, does not modulate γ-cleavage of β-CTF.

Inhibiting β-, but not γ-, cleavage of APP rescues the LTP deficit of FDD_{KI} mice

Long-term potentiation (LTP), a synaptic plasticity phenomenon that underlies memory, is defective in the hippocampal Schaffer collateral pathway of FDD_{KI} mice. To examine the effect of MoBA on LTP, hippocampal slices were perfused with MoBA for 60 min before inducing LTP. Both at 1 μM or 10 nM concentrations MoBA reversed the LTP deficit of FDD_{KI} samples and did not alter LTP in WT mice (Fig 3A). N6, which does not inhibit APP processing (Fig 2A), did not rescue LTP of FDD_{KI}

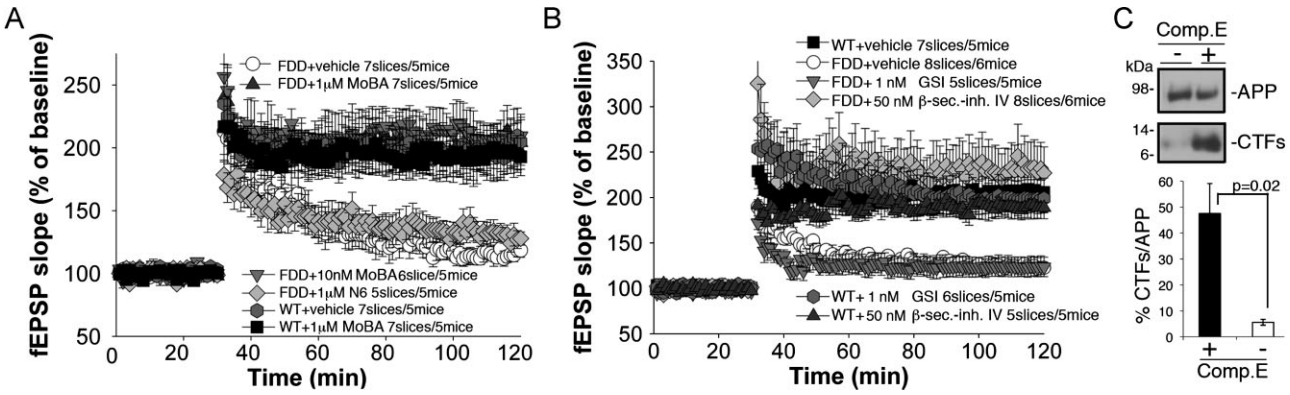

Figure 3. MoBA and a β-secretase inhibitor rescue the LTP deficit of FDD_{KI} mice—a GSI does not.

A. Sixty-minutes perfusion with MoBA reverses LTP impairment in FDD_{KI} mice [WT to FDD_{KI}: $F(1,12) = 12.372$, $p = 0.004$; WT to FDD_{KI} + MoBA 1 μM: $F(1,12) = 0.012$, $p = 0.914$; WT to FDD_{KI} + MoBA 10 nM: $F(1,11) = 0.202$, $p = 0.662$; FDD_{KI} to FDD_{KI} + MoBA 1 μM: $F(1,12) = (10.078)$, $p = 0.006$; FDD_{KI} to FDD_{KI} + MoBA 10 nM: $F(1,11) = 15.049$, $p = 0.008$]. N6 does not rescue the LTP deficit [FDD_{KI} to FDD_{KI} + N6 1 μM: $F(1,10) = 0.053$, $p = 0.821$]. MoBA does not alter LTP of WT mice [WT to WT + MoBA 1 μM: $F(1,12) = 0.361$, $p = 0.560$].

B. β-secretase-inhibitor IV (50 nM; $IC_{50} = 15$ nM) rescues LTP impairment in FDD_{KI} mice [FDD_{KI} to FDD_{KI} + β-secretase-inhibitor IV: $F(1,14) = 12.258$, $p = 0.004$; WT to FDD_{KI}: $F(1,13) = 12.272$, $p = 0.004$; WT to FDD_{KI} + β-secretase-inhibitor IV: $F(1,13) = 0.604$, $p = 0.451$]. There was a trend towards increased LTP in inhibitor IV-treated WT and FDD_{KI} samples versus vehicle-treated WT controls, but this difference was not statistically significant. Compound-E (1nM; $IC_{50} = 300/240$pM) does not rescue the LTP defect in FDD_{KI} samples [FDD_{KI} to FDD_{KI} + compound-E: $F(1,11) = 0.838$, $p = 0.380$]. The β- and GSIs do not alter LTP of WT mice [WT to WT + β-secretase-inhibitor IV: $F(1,10) = 0.413$, $p = 0.535$; WT to WT + compound-E: $F(1,11) = 0.041$, $p = 0.844$].

C. Lysates from hippocampal slices treated with (+) or without (−) compound-E for 3 h, were analysed by WB for APP and CTFs. The bottom graph represents quantization of triplicate samples. The CTFs levels are expressed as a % of APP.

mice (Fig 3A). Since perfusing hippocampal slices with a 50 nM concentration of β-secretase-inhibitor IV for 1 h before inducing LTP exhibited the same effect of MoBA (Fig 2B), it is reasonable to conclude that MoBA ameliorates LTP of FDD_{KI} mice by inhibiting β-cleavage of APP and not by unrelated mechanisms.

Inhibition of β-cleavage of APP could rescue LTP preventing Aβ production, which is considered the primary mediator of synaptic abnormalities in AD. However, inhibition of γ-secretase by perfusing hippocampal slices with a 1 nM concentration of the GSI compound-E for 1 h before inducing LTP did not ameliorate synaptic plasticity deficits of FDD_{KI} samples (Fig 3B). We verified that compound-E was active by measuring APP-CTFs in the hippocampal slices, which increased when γ-secretase was inhibited. APP-CTFs levels were significantly increased in samples treated for 3 h with compound-E as compared to untreated samples (Fig 3C). Therefore, it is very unlikely that γ-secretase-derived APP metabolites (such as Aβ, P3 and AID, Fig 1A) underlie this synaptic plasticity abnormality in FDD_{KI} mice. Overall, these findings indicate that β-cleavage of APP during LTP prompts the synaptic plasticity deficits of FDD_{KI} mice and suggest that *de novo* produced sAPPβ and/or β-CTF and not Aβ, are the synaptic-toxic APP species.

Inhibiting β-, but not γ-, cleavage of APP rescues the memory deficit of FDD_{KI} mice

We next tested the role of APP processing in the aging-dependent memory deficits of FDD_{KI} mice (Tamayev et al, 2010b). A cannula was surgically implanted in the lateral ventricle of a cohort of 8-month-old FDD_{KI} mice and WT littermates. Five weeks after surgery, we analysed the effect of β-secretase-inhibitor IV, MoBA and GSI on the memory deficits of FDD_{KI} mice in a longitudinal study. Memory was analysed using novel object recognition (NOR), a non-aversive memory test that relies on the mouse's natural exploratory behaviour. Before the NOR tests, open field studies showed that FDD_{KI} mice have no defects in habituation, sedation, risk assessment and anxiety-like behaviour in novel environments, as previously reported (Tamayev et al, 2010b). The first NOR study showed that during training, FDD_{KI} and WT mice spent the same amount of time exploring two identical objects (Fig 4A). The following day, one of the two old objects was replaced with a new one to test the mouse's memory. WT mice preferentially explored the novel object; conversely FDD_{KI} mice spent the same amount of time exploring the two objects as if they were both novel to them, showing that they had no memory of the objects from the previous day (Fig 4B). Two weeks later, we tested the effect of β-secretase-inhibitor IV. The mice were injected in the lateral ventricle with 1 μl of a 100 μM solution of β-secretase-inhibitor IV in PBS 1 h before the training/testing trials. Treated FDD_{KI} mice spent significantly more time exploring the novel object just as β-secretase-inhibitor IV-treated controls (Fig 4B). Following 1 day of rest, a new NOR test performed without treatments showed that FDD_{KI} mice had relapsed into amnesia (Fig 4B), demonstrating that the therapeutic effect of β-secretase inhibition is reversible and short-lived. Four days later, we analysed the behavioural outcome of γ-secretase inhibition. Mice were injected 1 h before the training/testing with 1 μl of a 300 nM solution of compound-E in PBS, The GSI neither improved memory of FDD_{KI} mice nor altered performance of

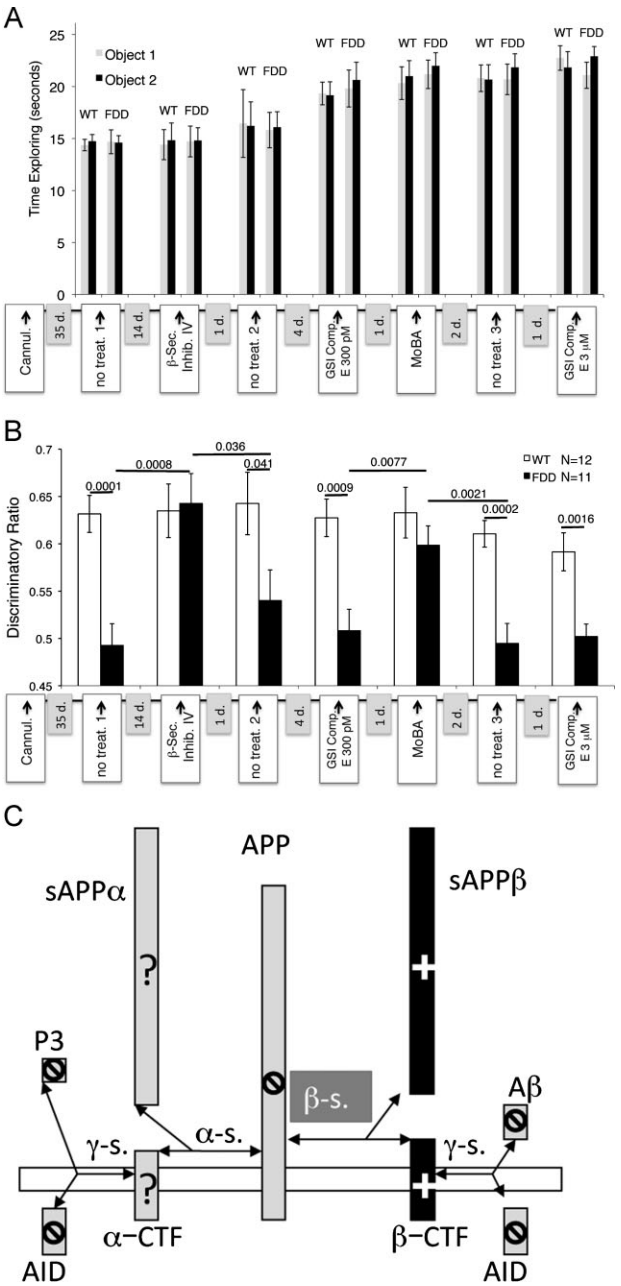

Figure 4. Inhibiting β-cleavage of APP rescue the memory deficit of FDD_{KI} mice. Mice were injected in the lateral ventricle with either 1 μl of PBS/100 μM β-secretase-inhibitor IV, 1 μl of PBS/300 nM compound-E, 1 μl of PBS/100 μM-MoBA or 1 μl of PBS/3 μM compound-E. Injections were performed 1 h prior to the training section and, the following day, 1 h before testing.

A. WT and FDD_{KI} mice spent the same amount of time exploring the two identical objects on day 1. As the mice develop habituation to the test, they tend to explore the objects more.

B. WT mice spent more time exploring the novel object 24 h later, showing normal object recognition (discriminatory ratio = 0.63), while FDD_{KI} mice present amnesia and do not distinguish the new object from the old one (discriminatory ratio = 0.5). β-secretase-inhibitor IV and MoBA transiently rescue this memory deficit, while GSI does not. The number of days between the day 2 of a test and day 1 of the following test are indicated (× d.).

C. Model depicting early pathogenic events preceding amyloidosis and leading to memory loss. It is unlikely that full-length APP is pathogenic since either decreasing (Tamayev et al, 2011) or increasing (MoBA and inhibitor IV) its levels prevents/rescues the deficits of FDD_{KI} mice. Two inhibitors of β-cleavage of APP (Inhibitor IV and MoBA), but not a GSI, rescue the LTP/memory deficits, suggesting that newly synthesized sAPPβ and/or β-CTF, but not Aβ/P3/AID cause these deficits in FDD_{KI} mice (+ and in black). Whether sAPPα and/or α-CTF are pathogenic remains to be determined (?).

WT animals (Fig 4B). Two days later, we assessed the therapeutic potential of MoBA by injecting in the lateral ventricle 1 μl of a 100 μM solution of MoBA in PBS 1 h before the training/testing sections. MoBA significantly improved memory in FDD_{KI} mice. Like for β-secretase-inhibitor IV, the therapeutic effect of MoBA was transitory. In fact, when this cohort of mice was retested after 48 h of rest and without treatments, we found that FDD_{KI} mice had reverted to a memory-loss phenotype (Fig 4B). To exclude that compound-E was ineffective due to low-dosage, mice were rested for 1 day and retested injecting 1 μl of a 3 μM solution of compound-E in PBS 1 h before the training/testing tasks. Even this 10-fold higher GSI dose did not correct the memory deficit of Danish mice, and GSI-treated WT mice showed a trend, though not statistically significant, towards memory impairment (Fig 4B). Thus, consistent with the LTP data, β-secretase-inhibitor IV and MoBA rescued, albeit temporarily, the memory deficit of FDD_{KI} mice, while the GSI did not.

DISCUSSION

Our findings demonstrate that the synaptic plasticity and memory deficits in FDD are mediated through production of sAPPβ and/or β-CTF during LTP and memory acquisition. Interestingly, it has been suggested that an APP fragment derived from sAPPβ contributes to AD pathogenesis acting via DR6 (Nikolaev et al, 2009). The failure of GSI to rescue the deficits of FDD_{KI} mice suggests that metabolites derived from γ-cleavage of APP, such as Aβ, P3 and AID, are not involved in these pathogenic processes (Fig 4C). Alternatively, the GSI may have a beneficial effect on LTP/memory deficits, possibly due to a reduction in γ-secretase-derived APP fragments, that is counterbalanced and masked by increased levels of toxic β-CTF caused by inhibition of γ-cleavage of APP (Fig 3C). Regardless, the data imply that inhibition of γ-processing of APP, even using γ-secretase modulators that inhibit APP cleavage sparing γ-processing of other substrates, may be therapeutically ineffective in correcting memory deficits and, perchance, harmful on account of the increase in β-CTF.

Considering that MoBA and β-secretase-inhibitor IV were injected only 1 h before the training/testing for the behavioural tests or perfused for 60 min prior to LTP measurements, it's hard to imagine that they could have manifestly affected the total

hippocampal levels of proteolytic products of APP. A logical interpretation of these results is that MoBA and β-secretase-inhibitor IV exert a therapeutic effect by reducing β-cleavage of APP, perhaps in the synaptic cleft, during synaptic events leading to LTP and memory acquisition. The temporarily therapeutic efficacy of β-secretase-inhibitor IV and MoBA in this longitudinal study supports this hypothesis, and suggest that, after the drugs are cleared and APP processing by β-secretase is restored at pathological levels, memory acquisition is impaired and the mice relapse into amnesia.

FDD_{KI} mice are genetically congruous to the human disease, suggesting that the mechanisms underlying synaptic and memory impairments in FDD_{KI} mice faithfully reproduce the pathogenesis of FDD. The inference that Aβ may not cause synaptic and memory dysfunction in FDD_{KI} mice is at odds with the belief that Aβ is the primary mediator of AD-related dementias. Perhaps, FDD_{KI} mice model early pathogenic events leading to memory loss in human dementia that precede amyloid lesions and tauopathy, while Aβ might play a role in later disease-stages. Although our data provide no evidence supporting a role for amyloid peptides in synaptic plasticity and memory deficits, amyloid plaques and/or 'toxic amyloid conformers' may set off other clinical symptoms of FDD/AD patients. It is also possible that the pathophysiology of FDD and AD are distinct and that Aβ is the primary cause of AD but not FDD. However, several analogies exist between FDD and FAD. FDD and most FAD cases are caused by loss of function mutations of genes that regulate APP processing [*BRI2/ITM2b* (Tamayev et al, 2010a; Tamayev et al, 2010b) and *PSEN1/PSEN2* (De Strooper, 2007; Saura et al, 2004; Shen & Kelleher, 2007; Zhang et al, 2009)]. It is worth noting that loss of function induced by *PSEN1* and *PSEN2* FAD mutations may cause, perhaps transiently during synaptic transmission and memory acquisition, an increase in the levels of APP-CTFs including β-CTF. In addition, FDD and FAD have common pathological and clinical presentation. Indeed, FDD presents all the hallmarks of AD. These genetic and clinical similarities between FDD and FAD strongly argue that they share common pathogenic mechanisms. It is important to mention that loss of BRI2 function results in increased processing of APP by both β- and α-secretases in FDD (Matsuda et al, 2011b; Tamayev et al, 2011). Although the data presented here do not address the role of the latter, it will be interesting to study whether the α-secretase-derived APP metabolites α-CTF and sAPPα are toxic, or whether α-processing of APP has a beneficial effect owing to the prevention of an even larger production of β-CTF/sAPPβ.

Overall, this study backs therapeutic approaches aiming to reduce sAPPβ/β-CTF levels, and suggest that targeting Aβ production and/or clearance is ineffective or, perhaps, detrimental. Since β-secretase has important biological functions (Hu et al, 2006; Hu et al, 2010; Kim et al, 2007; Willem et al, 2006) the use of a β-secretase inhibitor may produce adverse toxic effects, which would be avoided using compounds with a MoBA-like activity. Based on the mechanism of action, MoBA would also ameliorate symptoms and disease-stages mediated by Aβ.

MATERIALS AND METHODS

Cells, plasmids and reagents

Cells, transfection methods, APP expression construct and luciferase assays were described (Matsuda et al, 2005; Scheinfeld et al, 2002). BRI2 fragments were PCR-amplified and cloned into pcDNA3mycHisB (Invitrogen). The following antibodies were used: α-APP (22C11/Chemicon); α-sAPPα and α-sAPPβ (IBL); α-APPCTF (Invitrogen/Zymed); α-myc (Cell-Signaling); anti-α-Tubulin (Sigma); Flag-M2-agarose-beads (Sigma); secondary antibodies (Southern Biotechnology); β-secretase-Inhibitor IV and compound-E (Calbiochem); streptavidin-agarose-beads (Sigma).

β-secretase activity was tested using the Invitrogen FRET assay kit following the manufacturer's instructions.

BRI2-derived peptides and APP processing

APP-transfected HEK293 cells were incubated with the indicated peptides for 8 h. Peptides were used at either 25 μM (Fig 2A) or 5 μM (Fig 2B) concentration.

Precipitation with FLAG-peptides

To prepare brain cells, mouse brains were washed in PBS and minced in dissociation buffer. After sedimentation and filtration, dissociated cells were cultured in Neurobasal media. Cells incubated with Flagged-peptides were lysed and precipitated with Flag-M2-agarose-beads as described (Matsuda et al, 2005). Bound proteins were eluted with 100 μg/ml of FLAG peptide.

Surface biotinylation

HeLa/APP cells were surface biotinylated with sulfo-NHS-SS-biotin and treated with reducing reagent as described (Matsuda et al, 2011a).

Electrophysiological analysis

Experiments were performed as previously described (Puzzo et al, 2008). Briefly, animals were sacrificed by cervical dislocation followed by decapitation. Hippocampi were quickly removed. Transverse hippocampal slices (400 μm) were transferred to a recording chamber where they were maintained at 29°C and perfused with artificial cerebrospinal fluid (ACSF) continuously bubbled with 95% O_2 and 5% CO_2. The ACSF composition was: 124 mM NaCl, 4.4 mM KCl, 1 mM Na_2HPO_4, 25 mM $NaHCO_3$, 2 mM $CaCl_2$, 2 mM $MgSO_4$ and 10 mM glucose. For treatments with MoBA, β-secretase-Inhibitor IV and compound-E, hippocampal slices were perfused for 1 h prior to LTP induction with ACSF containing the indicated concentrations of these compounds. A 30 min baseline was recorded every min at an intensity that evokes a response ~35% of the maximum evoked response. LTP was induced using θ-burst stimulation (10 bursts repeated at 5 Hz, each one consisting of 4 pulses at 100 Hz). Responses were recorded for 2 h after tetanization and plotted as percentage of baseline field-excitatory-post-synaptic potentials (fEPSPs) slope.

Brain cannulation

The cannulas were surgically implanted by Dr. Xiaosong Li at the Animal Physiology core of the Albert Einstein College of Medicine. Compounds were injected using a CMA 400 syringe pump. Solutions were delivered at the rate of 1 μl per minute.

The paper explained

PROBLEM:

Mouse models are critical to explore pathogenesis and therapy of human diseases. Inadequate models are a major handicap for understanding disease mechanisms and for developing disease-modifying agents. The animal models used to study Alzheimer's disease are designed on the assumption that amyloid-β (Aβ) plaques cause dementia (Amyloid Cascade Hypothesis). These models consist of transgenic mice over-expressing mutant forms of genes causing human dementia because over-expression is required to develop amyloid lesions in mice. These mice however do not reflect the genotypes of human diseases and it is dubious whether they recapitulate the physiopathology of dementia. Indeed, over-expression of human disease genes could produce artefactual harmful effects, leading to erroneous information concerning the pathogenesis and therapy of dementia.

RESULTS:

To avoid many potential artefacts of the over expression models, we have produced a mouse model of human dementia genetically congruous to the human case. These mice develop memory and synaptic plasticity deficits that are dependent on Aβ precursor protein (APP). APP is cleaved by β-secretase, which produces two APP-metabolites called sAPPβ and β-CTF; β-CTF is than processed by γ-secretases to produce Aβ. We show here that synaptic plasticity and memory deficits are mediated through production of sAPPβ and/or β-CTF, but not Aβ, during LTP and memory acquisition. The inference that Aβ may not cause synaptic and memory dysfunction is at odds with the belief that Aβ is the primary mediator of AD-related dementias. Moreover we develop a biologic compound (modulator of β-cleavage of APP, MoBA) that blocks β-cleavage of APP but not β-secretase. Since β-secretase has important biological functions the use of a β-secretase inhibitor may produce adverse toxic effects, which would be avoided using compounds with a MoBA-like activity.

IMPACT:

Our study provides new insights into the pathogenic mechanisms underlying memory loss in neurodegenerative processes and suggest that memory deficits are primarily caused by APP metabolites distinct from Aβ. These findings have important clinical implications as they offer an alternative explanation to why anti-Aβ therapies in humans have so far failed, and advise against targeting γ-secretase cleavage of APP and/or Aβ in AD therapy. In addition, this work describes a lead compound with a novel mechanisms-of-action (MoBA), which could be a candidate for the therapeutic treatment of dementia.

Open field and novel object recognition

The mice were acclimated to the testing room for 30 min after being moved. Each mouse was placed into a 40 cm × 40cm open field chamber with opaque walls, 2 ft high. Each mouse was allowed to habituate to the normal open field box for 10 min, and repeated again 24 h later, in which the video tracking system (HVS 2020; HVS Image) quantified various locomotor parameters: total distance travelled, number of entries into, distance travelled in, and time spent in the centre of the locomotor arena. As previously reported (Tamayev et al, 2010b), open field studies showed that FDD_{KI} mice have no defects in habituation, sedation, risk assessment and anxiety-like behaviour in novel environments.

Novel object recognition began 24 h after the second open field session, and consisted of two sessions, 24 h apart. In the first session, the mice were placed into the open field chamber with two identical, non-toxic objects, 12 cm from the back and sidewalls of the open field box, and 16 cm apart from each other. A 10 min session, in which the time exploring each object was recorded; an area 2 cm^2 surrounding the object is defined such that nose entries within 2 cm of the object were recorded as time exploring the object. The animal was then returned to its home cage, and 24 h later, placed into the open field box again. This time, there were two new objects, one identical to the previous objects, and one novel object. The mice were given another 10 min to explore, and the amount of time exploring each object was recorded. Mice that spent <7 s exploring the objects were omitted from the analysis (Bevins & Besheer, 2006). Results were recorded as an object discrimination ratio (ODR), which is calculated by dividing the time the mice spent exploring a novel object, divided by the total amount of time exploring the two objects.

Statistical analysis

All data are shown as mean ± s.e.m. Statistical tests included two-way ANOVA for repeated measures and *t*-test when appropriate.

Ethical statement regarding the use and well fare of mice

Mice were handled according to the Ethical Guidelines for Treatment of Laboratory Animals of Albert Einstein College of Medicine. The procedures were described and approved in animal protocol number 20040707.

Author contributions

LD generated the FDD_{KI} mice; RT performed LTP, NOR and purified β-secretase activity experiments; LD designed the peptides; LD and SM performed biochemical experiments with peptides and transfection experiments; OA provided equipment for LTP experiments; LD, RT and SM analysed the data; LD designed research and wrote the paper.

Acknowledgements

This work was supported by grants from the Alzheimer's Association (IIRG-09-129984), the Edward N and Della L. Thome Memorial Foundation grant and the National Institutes of Health (NIH; R01AG033007) to LD.

Conflict of interest statement: The FDD_{KI} mice and the MoBA peptide are patented by the Albert Einstein College of Medicine. RT, SM and LD are inventors of these patents.

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