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Expression of human β-secretase in the mouse brain increases the steady-state level of β-amyloid

Ursula Bodendorf, Simone Danner, Frauke Fischer, Muriel Stefani, Christine Sturchler-Pierrat, Karl-Heinz Wiederhold, Matthias Staufenbiel and Paolo Paganetti

Nervous System, Novartis Pharma AG, Basel, Switzerland

Abstract

β-Site APP-cleaving enzyme (BACE) initiates the processing of the amyloid precursor protein (APP) leading to the generation of β-amyloid, the main component of Alzheimer's disease senile plaques. BACE (Asp2, memapsin 2) is a type I transmembrane aspartyl protease and is responsible for the β-secretase cleavage of APP producing different endoproteolytic fragments referred to as the carboxy-terminal C99, C89 and the soluble ectodomain sAPPβ. Here we describe two transgenic mouse lines expressing human BACE in the brain. Overexpression of BACE augments the amyloidogenic processing of APP as demonstrated by decreased levels of full-length APP and increased levels of C99 and C89 *in vivo*. In mice expressing huBACE in addition to human APP wild-type or carrying the Swedish mutation, the induction of APP processing characterized by elevated C99, C89 and sAPPβ, results in increased brain levels of β-amyloid peptides Aβ40 and Aβ42 at steady-state.

Keywords: β-amyloid, BACE, protein processing, transgenic mice.

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Alzheimer's disease (AD) is a progressive dementia characterized by the deposition of fibrillar β-amyloid (Aβ) in the brain parenchyma and vasculature (for a review see Selkoe 2001). It is unclear which mechanisms lead to the deposition of Aβ. In early onset forms of AD, mutations in the genes encoding the β-amyloid precursor protein (APP) or the presenilins (PS) cause an elevation of total Aβ or the relative increase of longer forms of Aβ, which deposit more readily (Goate *et al*. 1991; Sherrington *et al*. 1995). Accordingly, the expression of mutated APP in transgenic mice results in the appearance of AD-like amyloid plaques, a process accelerated by the coexpression of PS carrying familial Alzheimer's disease mutations (Games *et al*. 1995; Duff *et al*. 1996; Hsiao *et al*. 1996; Sturchler-Pierrat *et al*. 1997). However, the majority of AD patients lack these mutations. In these cases, the disease may be caused by alterations in Aβ generation, by factors favouring Aβ fibrillation or by impaired Aβ clearance. Recent studies have indeed shown that Aβ deposition can be modulated by immunization with synthetic Aβ, by TGF-β1 or by neprilysin (Schenk *et al*. 1999; Iwata *et al*. 2001; Wyss-Coray *et al*. 2001). The inhibition of Aβ generation by interference with the proteolytic activities that regulate APP processing to Aβ, i.e. β-secretase and γ-secretase, is expected to diminish the concentration of Aβ in the brain and ultimately impair Aβ deposition. Indeed, a marked reduction in brain Aβ levels is observed after acute treatment of APP transgenic mice with a peptidomimetic inhibitor of γ-secretase activity (Dovey *et al*. 2001). The molecular identity of the catalytic polypeptide is controversial but γ-secretase is thought to be a large oligomeric complex with PS representing a requisite constituent.

BACE (Asp2, memapsin 2) was identified as a type I transmembrane aspartic protease expressed in brain neurones (Hussain *et al*. 1999; Sinha *et al*. 1999; Vassar *et al*. 1999; Yan *et al*. 1999). It satisfies the requirements anticipated for β-secretase. Notably, the ablation of the gene encoding BACE completely impairs β-secretase cleavage of APP and

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Address correspondence and reprint requests to Paolo Paganetti, WSJ.386.828, Novartis Pharma AG, CH-4002 Basel, Switzerland. E-mail: paolo.paganetti@pharma.novartis.com

Abbreviations used: Aβ, β-amyloid pepetide; AD, Alzheimer's disease; APP, β-amyloid precursor protein; APP_{SWE}, APP carrying the Swedish mutation; BACE, β-site APP-cleaving enzyme; C99, the C-terminal 99 amino acid intermdiate generated by BACE; C89, the alternative C-terminal amino acid intermidiate generated by BACE.

abolishes the generation of Aβ (Cai *et al*. 2001; Luo *et al*. 2001; Roberds *et al*. 2001). The BACE knock-out mice develop normally and show no phenotypic alterations. In cell culture, Aβ generation is increased by BACE overexpression (Hussain *et al*. 1999; Sinha *et al*. 1999; Vassar *et al*. 1999; Yan *et al*. 1999) and decreased by the application of antisense oligonucleotides specific for the BACE mRNA (Vassar *et al*. 1999; Yan *et al*. 1999). BACE also cleaves amino-terminally to Glu11 of Aβ. This alternative cleavage leads to the production of C89 from which Aβ can no longer be generated. So far the effect of BACE overexpression has not been analysed *in vivo*. Using transgenic mice, we show that the overexpression of huBACE in neuronal cells of the brain also promotes the amyloidogenic processing of APP *in vivo*. In young huBACE × huAPP bigenic mice, despite a robust increase in C89, BACE overexpression induces the amyloidogenic processing of APP and elevates the steady-state levels of Aβ1–40 and Aβ1–42 peptides. It appears that the modulation of BACE activity is sufficient to alter Aβ levels in the brain, a process expected to affect Aβ plaque formation.

Experimental procedures

Transgenic mice

The cDNA constructs used to generate the transgenic mouse lines analysed in this study contain a murine Thy-1 expression cassette (Andrä *et al*. 1996). The BACE54 construct was obtained by insertion of the complete huBACE cDNA (Bodendorf *et al*. 2001) into the filled-in *Xho*I site of the vector pTSCα1. APP51/22 mice were obtained with a construct containing the human wild-type APP751 cDNA subcloned in the *Xho*I restriction site of the same vector. The generation of the APP23 transgenic mice, which express the Swedish variant of APP, was described elsewhere (Sturchler-Pierrat *et al*. 1997). While APP23 mice were generated by pronuclear injection of B6D2F1 × B6D2F1 embryos and backcrossed to C57BL/6 J for more than 10 generations, the other mouse lines were generated in the C57BL/6 J background. Mice were sacrified at 2–3 months of age.

All animal manipulations were performed in full accordance to the guidelines released by the responsible Swiss veterinary office.

In situ hybridization

In situ-hybridization was performed on 10-μm cryostat-sections of mouse brains using a riboprobe. For the construction of the riboprobe, a fragment of huBACE (position 815–1593 of the open reading frame) was amplified by standard polymerase chain reaction from a human brain cDNA library (Clontech, Palo Alto, CA, USA). Primers used were flanked by the T7-promoter at the 5′-end and the SP6-promoter at the 3′-end. *In vitro* transcription and [^{35}S]-radioactive labelling was performed with the RNA Transcription Kit (Stratagene, La Jolla, CA, USA) according to the manufacturer's protocol. For the hybridization reaction, each slide was incubated with total 10^6 cpm labelled riboprobe following standard conditions as described earlier (Sola *et al*. 1993) and exposed to β-max film (Amersham Pharmacia Biotech, Uppsala, Sweden) for 4 weeks.

Brain homogenates, immunoprecipitation from brain tissue and western blot analysis

We have used wet-weight, total protein or tubulin content of brain samples for normalization. The most robust results were obtained considering the wet-weight, which we therefore use routinely. For the wet-weight procedure, half brains were weighted and homogenized in 10 volumes of homogenization buffer (50 mM Tris–HCl pH 8.0, 100 mM sodium chloride, 5 mM EGTA, 5 mM dithiothreitol, 1 mM magnesium chloride, 1% NP-40, 1 mL buffer per 100 mg brain tissue) containing protease inhibitors (Roche Molecular Biochemicals, Rotkreuz, Switzerland). Before immunoprecipitation, the samples were cleared by centrifugation at 4°C for 15 min at 10 000 *g*. Proteins were immunoprecipitated using rabbit antiserum APPC8 raised against the carboxy-terminal amino acids of APP (Schrader-Fischer and Paganetti 1996) or monoclonal antibody β1 reacting to the amino-terminus of Aβ (Schrader-Fischer and Paganetti 1996) and protein A-sepharose (Amersham Pharmacia Biotech). Precipitates or whole homogenates were separated either on 13% Tris–bicine gels (carboxy-terminal metabolites), 10% Tris–bicine gels with 8 M urea (Aβ) or 8% Tris–glycine Laemmli gels (APP, sAPPβ) as described (Klafki *et al*. 1996; Schrader-Fischer and Paganetti 1996). Proteins were then transferred to Immobilon-P membranes (Millipore, Bedford, MA, USA), probed with the indicated primary antibodies and detected by chemiluminescence (ECL, Amersham Pharmacia Biotech). Rabbit antisera 815 and 818 against the amino- and the carboxy-terminus of huBACE were described previously (Bodendorf *et al*. 2001). The antibodies 815 (against the amino acids 46–61 of human BACE501) and 818 (against amino acids 484–501) were affinity purified using the corresponding peptides (Bodendorf *et al*. 2001). Both antibodies react equally well with human as well as mouse BACE expressed in cultured mammalian cells (not shown). The neoepitope rabbit antisera specific to the carboxy-terminus of sAPPβ wild-type or carrying the Swedish mutation were raised against the synthetic peptides Cys-Ile-Ser-Glu-Val-Lys-Met (antiserum 879) and Cys-Ile-Ser-Glu-Val-Asn-Leu coupled to ovalbumine (antiserum 852), respectively. Protein amounts were determined using the software MCID V4.0 (Imaging Research Inc., St Catharines, ON, Canada) with densitometry scans of the western blots.

Phosphatase treatment of the APP carboxy-terminal fragments

Either whole brain homogenates or APP carboxy-terminal fragments precipitated with APPC8 were digested with 600 U of λ protein phosphatase (New England Biolabs, Beverly, MA, USA) for 15 min at 30°C in 50 mM Tris–HCl pH 7.5, 0.1 mM EDTA, 5 mM dithiothreitol, 2 mM mangane chloride and 0.01% Brij 35. The λ protein phosphatase acts on serine, threonine and tyrosine residues.

Statistical analysis

The data are reported as means ± SEM in percentages. Values are calculated by setting arbitrarily the values obtained in control

animals, i.e. non-transgenic mice for huBACE, as 100%. For statistical analysis the one tailed Student's *t*-test was used.

Results

Overexpression of huBACE in brains of two transgenic mouse lines

To analyse how BACE overexpression affects the processing of APP *in vivo*, we have generated the two transgenic mouse lines BACE54/4 and BACE54/11 that express the cDNA encoding human BACE501 (huBACE) at high levels in the brain. To this end, we have used the murine Thy-1 promoter, which is known to drive efficient neural expression of several proteins including human APP (Andrä *et al*. 1996; Sturchler-Pierrat *et al*. 1997). *In situ* hybridization using line BACE54/4 revealed expression of the huBACE mRNA in the gray matter of neocortex and hippocampus as shown on sagittal brain sections (Fig. 1a). The expression in the caudate putamen, brain stem, thalamus and, in particular, the cerebellum was lower. In control sections obtained from wild-type mice, a weak signal was detected in the hippocampus, cortex and cerebellum (Fig. 1a), consistent with the distribution of endogenous BACE mRNA described previously (Vassar *et al*. 1999). No signal was observed when a sense probe was used as negative control (Fig. 1a). By comparing the optical density of the sections, we conclude that overall the levels of the transgenic human BACE mRNA was approximately fourfold higher than that of the endogenous BACE mRNA. On the protein level, expression of huBACE was demonstrated in total brain homogenates of transgenic animals using polyclonal antisera specifically reacting with the amino-terminus (Fig. 1b) and the carboxy-terminus of BACE (not shown). Mouse brain-derived huBACE migrates on polyacrylamide gels as a diffuse band with a relative molecular mass of about 70 kDa due to extensive glycosylation of the catalytic ectodomain of BACE (Bodendorf *et al*. 2001; Charlwood *et al*. 2001). Consistently high levels of huBACE were observed in all analysed animals of the BACE54/4 line. However, substantial variation in BACE protein expression was observed between individual animals of the BACE54/11 line. In two out of seven mice the expression of huBACE was very high, whereas in the other animals the expression was below that of the BACE54/4 mice (Fig. 1b). Endogenous mouse BACE was not detected under these conditions. This was not due to lack of cross-reactivity of the antibodies with human and mouse BACE but rather to the very low levels of endogenous BACE, which could not be detected when total brain homogenates were analysed (Vassar *et al*. 1999; Bodendorf *et al*. 2001). The analysis of distinct brain regions confirmed the results obtained by *in situ* hybridization showing highest expression of the protein in the cortex and hippocampus and lowest expression in the cerebellum (Fig. 1c).

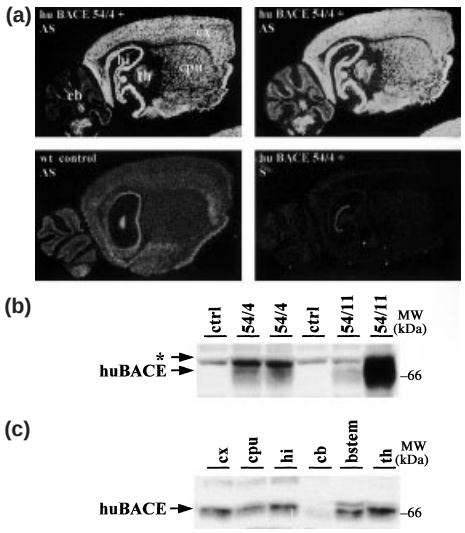

Fig. 1 huBACE transgenic mice: expression of mRNA and protein in the brain. (a) *In situ* hybridization of the huBACE mRNA on sagittal brain sections of a BACE54/4 mouse and of a wild-type mice using an antisense (AS) and a sense (S) probe as indicated. To better visualize the distribution of the transgene huBACE mRNA a shorter and a longer exposition of the hybridization of the BACE54/4 sections are shown. The identical long exposition was used for the sections of the wild-type and for the negative control sense probe. (b) Immunoblot of huBACE (antibody 815) in total brain homogenates prepared from BACE54/4, BACE54/11 and control mice. Human BACE501 was detected as a diffuse band at 70 kDa. The relatively large apparent molecular weight and the diffuse pattern, which were identical to those observed for human (and mouse) BACE expressed in mammalian cells (not shown), appeared to be caused by extensive glycosylation of the ectodomain. Endogenous mouse BACE was not detected in total brain homogenates. The asterisk denotes an unspecific reaction of the antibody 815 to a protein present in total brain homogenates. This protein was not recognized by the antibody 818, does not co-migrate with recombinant human or mouse BACE expressed in mammalian cells and it is not glycosylated (not shown). (c) Immunoblot of huBACE in samples obtained from distinct brain regions of a BACE54/4 mouse. cx, cortex; cpu, caudate putamen; hi, hippocampus; cb, cerebellum; bstem, brain stem; th, thalamus. Molecular mass markers are shown in kDa.

Processing of endogenous mouse APP by huBACE

We determined the steady-state amount of APP present in brains of the huBACE mice by immunoblot analysis with an antibody specific for the carboxy-terminus of APP. Total full-length APP was significantly reduced in the transgenic mice to 83 ± 3% (BACE54/4) and 75 ± 5% (BACE54/11) of that present in the wild-type mice (Fig. 2a). BACE was shown to generate *in vitro* two APP carboxy-terminal fragments starting either at Asp1 (C99) or Glu 11 (C89) of the Aβ sequence (Vassar *et al*. 1999). However, five distinct bands were identified in the brain homogenates. These five protein bands can be reduced by dephosphorylation to three major

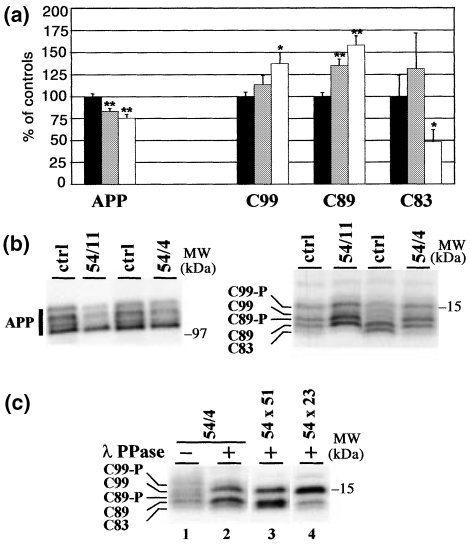

Fig. 2 Analysis of endogenous APP metabolism in BACE54/4 and BACE54/11 mice. (a) The amount of mouse full-length APP and carboxy-terminal fragments was determined by immunoblot analysis in brains of BACE54/4 (gray bars, $n = 7$) or BACE54/11 (white bars, $n = 8$) mice and compared with the levels determined in wild-type mice (black bars, $n = 9$) arbitrarily set to 100%. All values are given as means ± SEM. $^*p < 0.05$, $^{**}p < 0.01$. (b) Representative immunoblots of full-length APP and carboxy-terminal metabolites (APPC8 antibody) in wild-type and transgenic mice. Molecular mass markers are shown in kDa on the right. (c) Immunoblot of carboxy-terminal fragments of APP before (–) or after (+) λ protein phosphatase treatment present in wild-type, single or bigenic huBACE mice. For phosphatase treatment, the samples were either first immunoprecipitated with antibody APPC8 (lanes 1–2) or loaded directly (lanes 3–4).

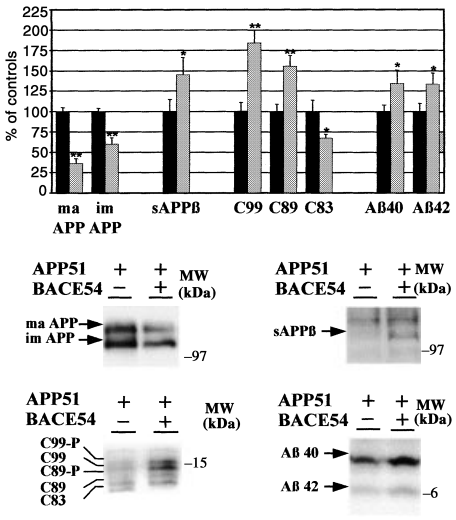

Fig. 3 Analysis of wild-type huAPP and metabolites in huBACE54/4 × APP51/22 mice. The changes in full-length huAPP751 and metabolites were determined from immunoblots of brain homogenates obtained from BACE54/4 × APP51/22 (gray bars, $n = 9$) or age-matched APP51/22 littermates (black bars, $n = 8$, set arbitrarily to 100%). Values (means ± SEM) are given as percentage of the respective metabolite in the APP51/22 mice. $^*p < 0.05$, $^{**}p < 0.01$. Representative immunoblots are shown on the lower panel.

protein fragments (Fig. 2c) in accordance with what was reported previously (Buxbaum *et al*. 1998). Their identity as C99, C89 and C83 was confirmed by co-migration, with reference peptides expressed in cultured cells (not shown). For quantification, the respective phosphorylated and non-phosphorylated carboxy-terminal fragments were not differentiated. Compared with the wild-type mice, a significant increase in C89 to 135 ± 7% (BACE54/4) and to 158 ± 11% (BACE54/11) was observed. The increase in C99 was statistically significant only in the BACE54/11 mice (137 ± 13%). In this line, but not in the BACE54/4 mice, C83 generated by α-secretase was reduced significantly to 48 ± 14% when compared with control animals. These data demonstrate that *in vivo* expression of huBACE promotes amyloidogenic processing of endogenous mouse APP in neurones of the central nervous system.

Processing of human APP in bigenic mice overexpressing huBACE and APP751

The contribution of human BACE to the processing of human APP was then studied in mice overexpressing huBACE (BACE54/4 line) as well as huAPP751 (APP51/22 line). The BACE54/4 mice were chosen because this line showed little variation in huBACE expression between the animals (see above). As shown in Fig. 3, in APP51/22 mice we detected two post-translationally modified forms of full-length huAPP carrying either N- and O-linked sugars or N-linked sugars only (mature and immature huAPP, respectively) as identified previously (Weidemann *et al*. 1989; Andrä *et al*. 1996). In the bigenic mice, overexpression of huBACE diminished the steady-state amount of both forms of huAPP when compared with the APP51 littermates (Fig. 3). Interestingly, the mature form was reduced more (to 36 ± 6%) than the immature form (to 60 ± 8%). The reduction of full-length huAPP was accompanied by a significant increase in the carboxy-terminal metabolites generated by β-secretase and in sAPPβ, the complementary metabolite of C99. In BACE54 × APP51 mice, C99 reached 184 ± 16% and C89 was elevated to 155 ± 14% when compared with the respective amounts in the APP51 mice. sAPPβ was increased to 145 ± 21%. HuBACE overexpression decreased the non-amyloidogenic processing of huAPP by α-secretase as judged by reduced C83 levels (67 ± 5%). To analyse the Aβ peptides, a western blot methodology optimized for the separation of Aβ1–40 from Aβ1–42 was used (Klafki *et al*. 1996). In this system, Aβ1–40 migrates slower than Aβ1–42 and at a larger apparent molecular weight. The results of this analysis demonstrated that the increased initiation of the amyloidogenic pathway by the presence of huBACE ultimately resulted in an elevated

steady-state amount of both Aβ1–40 (134 ± 17%) and Aβ1–42 (133 ± 14%).

Processing of the Swedish variant of APP in bigenic mice

Next, we analysed how huBACE expression affects the processing of the AD-linked Swedish variant of huAPP ($huAPP_{SWE}$) by crossing the BACE54/4 mice with the APP23 mice (Sturchler-Pierrat *et al*. 1997). *In vitro*, the $huAPP_{SWE}$ molecule was shown to be a better substrate for β-secretase due to the double missense mutation Lys-Met/Asn-Leu at the amino-terminus of the classical BACE cleaving site (Vassar *et al*. 1999). The APP23 mice have similar steady-state levels of huAPP as the APP51/22 animals (not shown). In the BACE54 × APP23 mice, the presence of huBACE significantly reduced full-length $huAPP_{SWE}$, too (Fig. 4). Similar to what we observed above, the mature form decreased to 29 ± 2% and the immature form to 84 ± 3% of the steady-state amount detected in the APP23 mice. In accordance, the huAPP metabolites generated by β-secretase were increased to 132 ± 5% for C99, to 179 ± 10% for C89 and to 140 ± 13% for sAPPβ. Again, the increase in amyloidogenic processing of $huAPP_{SWE}$ also resulted in elevated steady-state levels of the Aβ peptides. Aβ1–40 was enhanced to 132 ± 10% and Aβ1–42 to 151 ± 19%. In these bigenic mice, the α-secretase product C83 was not changed significantly.

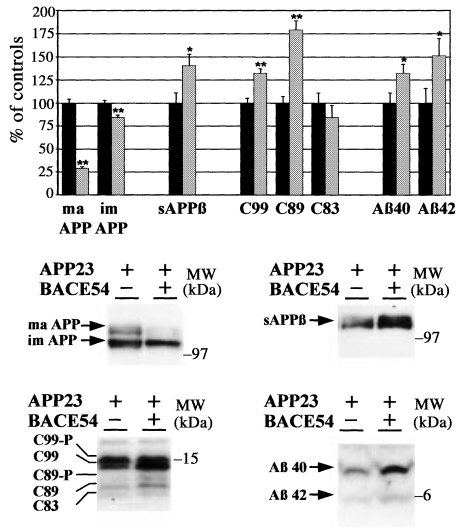

Fig. 4 Analysis of $huAPP_{SWE}$ and metabolites in huBACE54/4 × APP23 mice. The analysis was performed as described in the legend of Fig. 3 using bigenic BACE54/4 × APP23 mice expressing human APP carrying the Swedish mutation. BACE54/4 × APP23 (gray bars, $n = 12$) were compared with aged-matched APP23 littermates (black bars, $n = 12$). Values (means ± SEM) are given as the percentage of the respective metabolite in the APP23 mice. *$p < 0.05$, **$p < 0.01$. Representative immunoblots are shown on the lower panel.

Table 1 Relative levels of C99 and C89 in huAPP mice

	C99 (% ± SEM)	C89 (% ± SEM)
APP51/22	100 ± 9	101 ± 8
APP23	440 ± 20	111 ± 7
BACE54/4 × APP51/22	184 ± 16	157 ± 14
BACE54/4 × APP23	581 ± 21	199 ± 11

The values are in percentage. The amount of C99 determined in the APP51/22 mice was arbitrarily set to 100%.

Effect of the Swedish mutation in relation to huBACE overexpression

Finally, the change in the amyloidogenic processing of APP resulting from the overexpression of huBACE was related to that caused by the Swedish mutation. To this end, the amounts of the carboxy-terminal metabolites C99 and C89 in the mouse lines expressing huAPP were compared with the C99 levels detected in the APP51 mice which were set arbitrarily to 100% (Table 1). In the animals expressing wild-type huAPP, C99 and C89 were present at equal steady-state levels. The Swedish mutation in the APP molecule (APP23 mice) caused an increase in the amount of C99 corresponding to 440 ± 20% of that in the APP51 mice ($p < 0.01$). Remarkably, the amount of C89 was not altered significantly in the presence or absence of the Swedish mutation. In contrast, huBACE significantly enhanced the levels of C99 and C89 to 184 ± 16% and 157 ± 14%, respectively. It should be noted that the changes due to the expression of huBACE and to the presence of the Swedish mutation were not entirely additive with relatively lower levels for C99 and higher levels for C89 in the BACE54 × APP23 mice.

Discussion

We have generated two transgenic mouse lines expressing human BACE. *In situ* hybridization and western blot analysis demonstrated transgene expression in the brain with robust levels in the neocortex and hippocampus. The overexpression of BACE in neurones of the central nervous system, which are known to express APP, allowed the detailed analysis *in vivo* of the changes in metabolism of mouse and human APP caused by the presence of huBACE.

We report a significant reduction in the amount of full-length APP in both transgenic lines, a result that suggests increased metabolism of APP. Accordingly, C99 and C89, i.e. the processing products generated by β-secretase activity, were elevated. The most significant effect was the increase in C89 oberved in both mouse lines, whereas C99 was increased only in the huBACE54/11 line. We conclude that the overexpression of huBACE in the rodent brain favours increased amyloidogenic processing of APP. In addition, as the human enzyme appeared to process efficiently

endogenous mouse APP to C89 and C99, we did not observe a species-specific cleavage at either processing sites. This is in contrast to what was reported for cultured murine primary cortical neurones expressing human APP in the presence or absence of human BACE describing the species-specific production of C89 (Cai *et al*. 2001).

The analysis of bigenic mice expressing huBACE together with huAPP751 wild-type or carrying the Swedish mutation showed in both cases a significant reduction of the immature and mature forms of full-length APP. The most prominent effect was on the mature form. This is consistent with the concept that the processing of APP by huBACE may preferentially occur after completion of O-glycosylation in a post-Golgi compartment or in the endocytic pathway. The decrease of full-length APP was accompanied by a significant increase in the β-secretase cleavage products C89, C99 and sAPPβ. Increased amyloidogenic processing of APP led to the accumulation of Aβ40 and Aβ42 in the brain of young mice. Most studies performed using cultured cells have shown that BACE overexpression results in significant increase in BACE-mediated metabolites of APP including Aβ. The effects reported were not reproduced by all authors and varied over a wide range (Hussain *et al*. 1999; Sinha *et al*. 1999; Vassar *et al*. 1999; Yan *et al*. 1999). Our data indicate that *in vivo* the inititiation of the amyloidogenic processing of APP mediated by BACE leads to a significant increase in the steady-state concentration of Aβ. The modest increase in Aβ may be explained by the fact that in the brain the steady-state concentrations of Aβ are the result of the balance between the generation and the clearance rate. The observed similar increase in APP intermediates (C99 and sAPPβ) and Aβ indicates a direct relationship between the increase in amyloidogenic processing and the increase in Aβ. This suggests that *in vivo* the rate-limiting cleavage in the generation of Aβ is that regulated by BACE rather than the processing event mediated by γ-secretase.

Remarkably, in APP51 mice, the cleavage of APP generating C99 or C89 seemed to occur at the same efficiency as suggested by identical steady-state levels. At this point, we cannot exclude that APP may first be cleaved by BACE to generate C99, which is then subsequently cleaved at the amino-terminus of position Glu11 to yield C89. It was shown in cell culture that when C99 was offered as a substrate it was in fact cleaved by BACE at position Glu11 (Farzan *et al*. 2000; own unpublished results). To gain more information on this, we compared the changes in APP processing caused either by the overexpression of huBACE or by the Swedish mutation. As expected, the presence of the Swedish mutation provoked a robust increase in the steady-stade amount of C99. This is in accordance with the notion that the cleavage by BACE at position Asp1 of Aβ is more efficient in the optimized substrate carrying the Swedish mutation. However, as the levels of C89 were not changed despite the increase in C99, it is unlikely that C89 is generated from C99 when the Swedish variant of APP is offered as a substrate. Finally, we have found that the changes caused by overexpression of huBACE and those caused by the Swedish mutation were not additive. This result and the lack of interference with α-secretase cleavage observed in the BACE54 × APP23 mice suggest that in these mice BACE may process APP_{SWE} molecules that otherwise are destined for degradation. In contrast, the increase of C99 and the decrease in C83 in BACE54 × APP51 mice indicates that BACE may compete with α-secretase for the endoproteolytic cleavage of wild-type APP.

The involvement of BACE in the amyloidogenic cleavage of APP in cell culture has been well documented (Hussain *et al*. 1999; Sinha *et al*. 1999; Vassar *et al*. 1999; Yan *et al*. 1999). To analyse the changes in APP processing induced by BACE overexpression in neurones of the brain, we generated human BACE transgenic mice. We now demonstrate that the inititiation of the amyloidogenic pathway of APP processing by huBACE results in elevated steady-state levels of Aβ1–40 and Aβ1–42 in the brains of young transgenic mice. Our data support the view that BACE is the rate-limiting factor in the generation of Aβ. The formation of amyloid in the brain is known to depend on the steady-state levels of Aβ as indicated by the Swedish mutation of APP (Citron *et al*. 1994) as well as by studies with transgenic mice (Sturchler *et al*. 1997). An acceleration of amyloid deposition can therefore be expected when the huBACE mice age. An alternative mechanism leading to enhanced plaque deposition is the relative increase in Aβ1–42, the more amyloidogenic form of Aβ. AD-linked mutation in the presenilins act via this pathway (Selkoe 2001). It will be interesting to study how the increased brain load of Aβ peptides induced by the overexpression of huBACE will contribute to the onset and progression of plaque deposition in mice, in particular when compared with the transgenic mice expressing human APP and PS carrying familial AD mutations.

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