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Increase in Presenilin 1 (PS1) levels in senescence-accelerated mice (SAMP8) may indirectly impair memory by affecting amyloid precursor protein (APP) processing

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SUMMARY

Senescence-accelerated mice (SAMP8) serve as a model for Alzheimer's disease (AD) as they exhibit early loss of memory and increased amyloid precursor protein (APP) expression. APP is a ubiquitous membrane protein that is physiologically processed by site-specific proteolysis firstly by α- or β-secretases, releasing a large fragment called APP_S that contains most of the extracellular sequences of APP, a small extracellular stub, the transmembrane region and the cytoplasmic tail of APP ('AICD'-APP intracellular domain). These are subsequently cleaved by γ-secretase at multiple sites in the transmembrane region, releasing small peptides, $Aβ_{1-40}$ and $Aβ_{1-42}$, the major components of AD-associated amyloid fibrils. γ-secretase is a high-molecular-mass complex composed of presenilin-1 (PS1), nicastrin, APH-1 and Pen-2. As PS1 has been shown to play a critical role in facilitating γ-secretase activity, and mutations in this protein are associated with familial AD (FAD), we have cloned it from SAMP8 mouse hippocampus and compared its sequence with those of other species. Furthermore, changes in the expression of PS1 with age in the hippocampal tissue of SAMP8 were studied. The results showed that the SAMP8 PS1 cDNA sequence is identical to that of normal mice. However, its expression in the hippocampus of SAMP8 exhibited an increase, while CD-1 mice, a strain that does not exhibit premature memory loss, showed no change with age. An increased amount or mutation(s) in PS1, which alters the stoichiometric balance of the γ-secretase complex, may be the cause of aberrant or increased processing of APP, resulting in Aβ accumulation leading to loss of memory.

Key words: aging, amyloid, memory, presenilin, scenecence.

INTRODUCTION

Alzheimer's disease (AD) is a neurodegenerative disorder that is characterized by the presence of numerous senile plaques and neurofibrillary tangles (Goedert and Crowther, 1989). These are usually localized to the cortex and hippocampus and correlate with memory loss (Chen and Fernandez, 1999). Two types of AD have been described. Sporadic AD is a general deterioration of intra-neuronal contact without any association to any genetic element. Familial AD (FAD), on the other hand, is associated with mutations in amyloid precursor protein (APP) on chromosome 21, apolipoprotein E gene on chromosome 19, presenilin-1 (PS1) on chromosome 14 (14q24.3) and presenilin-2 (PS2) on chromosome 1. The majority of FAD early-onset is caused by mutations in the PS1 gene *inter alia* (Edwards-Lee et al., 2006; Pantieri et al., 2005). The mutations appear to give rise to types of proteins that have an increased tendency to form fibrils that associate with $Aβ_{1-42}$ and cause further aggregation, resulting in the formation of plaques typical of AD (Maury et al., 1997). PS1 is also required for proper processing of APP by γ-secretase, and mutations in PS1 in some way augment the aberrant processing of APP (Xia et al., 1998). Levels of γ-secretase modulate the $Aβ_{42}/Aβ_{40}$ ratio, which is important for plaque formation (Yin et al., 2007).

In view of the importance of PS1 in APP processing, we hypothesize that its levels play a significant role in the modulation of Aβ levels, thereby causing memory loss with age. SAMP8 mice, which exhibit early loss of memory, therefore serve as an excellent model for such study.

In the Golgi region, PS1 exists as a heterodimer, with the NTF (N-terminal fragment) and CTF (C-terminal fragment) separated but closely associated in a 1:1 stoichiometry of about 150 kDa (Capell et al., 1998). PS1 consists of 467 amino acids with a molecular mass of 52,671, arranged in 7–9 transmembrane (TM) helices and a hydrophilic acidic loop region encompassing amino acids 203–407. It is subjected to endolytic cleavage between amino acids 260 and 320, generating a 27–28 kDa NTF and a 17–18 kDa CTF derivative (Ratovitski et al., 1997; Thinakaran et al., 1996). In the endoplasmic reticulum, it exists as an inactive uncleaved 53 kDa holoprotein (Huynh et al., 1996). In the mouse brain, both the 150 kDa heterodimer (NTF–CTF associated complex) and the 53 kDa holoprotein can be detected (Capell et al., 1998). Immunohistochemical staining has revealed that PS1 is localized predominantly to large neurons in areas that have increased concentrations of senile plaques in AD, such as the hippocampal formation, entorhinal cortex and the subiculum (Huynh et al., 1996). Early on, PS1 was shown to bind APP at one or more sites (Waragai et al., 1997; Weidemann et al., 1997). Later, γ-secretase and associated enzymes were found to participate in cleaving APP to generate $Aβ_{42}$ (Evin et al., 2000; Wolfe et al., 1999).

For the current investigation, we have cloned PS1 from the hippocampus of SAMP8 mouse. Its sequence is compared with those

of other species, including the house mouse, in order to locate any mutations that may be presented in the SAMP8 mouse sequence. Many mutations that are linked to FAD occur in human PS1 but only a few occur in APP. Therefore, even a single change in the PS1 sequence in SAMP8 mouse may explain the cause of its loss of memory at an earlier age. While no change in sequence was noticed in the SAMP8 PS1, protein expression increased with age in these mice, providing a possible mechanism for the increase in hippocampal Aβ.

MATERIALS AND METHODS

Materials

All fine chemicals were obtained from Sigma Chemical Company, St Louis, MO, USA. Sequencing grade [^{35}S]ATP was obtained from ICN Biomedicals, Costa Mesa, CA, USA. PVDF membranes for western blotting were supplied by BioRad Laboratories, Hercules, CA, USA. Lipofectamine was supplied by BRL-GIBCO, Gaithersburg, MD, USA. Antibody that detects the PS1 holoprotein was obtained from Santa Cruz Biotechnology, Inc., Santa Cruz, CA, USA. GAPDH antibodies are from Calbiochem, San Diego, CA, USA. 4–12% Bis-tris gels with MES buffer were from Invitrogen, Carlsbad, CA, USA. Senescence-accelerated P8 line (SAMP8) and CD-1 mice were from our in-house colonies. Hippocampal tissues were dissected from the brains of euthanized mice after CO_2 administration. All animal protocols were performed in an AALCC (International Association for Assessment and Accreditation) accredited facility and approved by the animal committee of the VA and St Louis University Medical Centers.

RNA isolation and molecular cloning

Total RNA was isolated from the hippocampal tissue of 4-month-old P8 mice by a previously described method (Chirgwin et al., 1979). Cloning of presenilin cDNA was performed by RT-PCR from total RNA isolated from SAMP8 hippocampus. The primers used were 5′-ATGACAGAGATACCTGCACCTTTG–forward and 5′CTAGATATAAAACTGATGGAATGCAA–reverse. The PCR products from three independent reactions were directly cloned into the PCR-II vector supplied by Invitrogen.

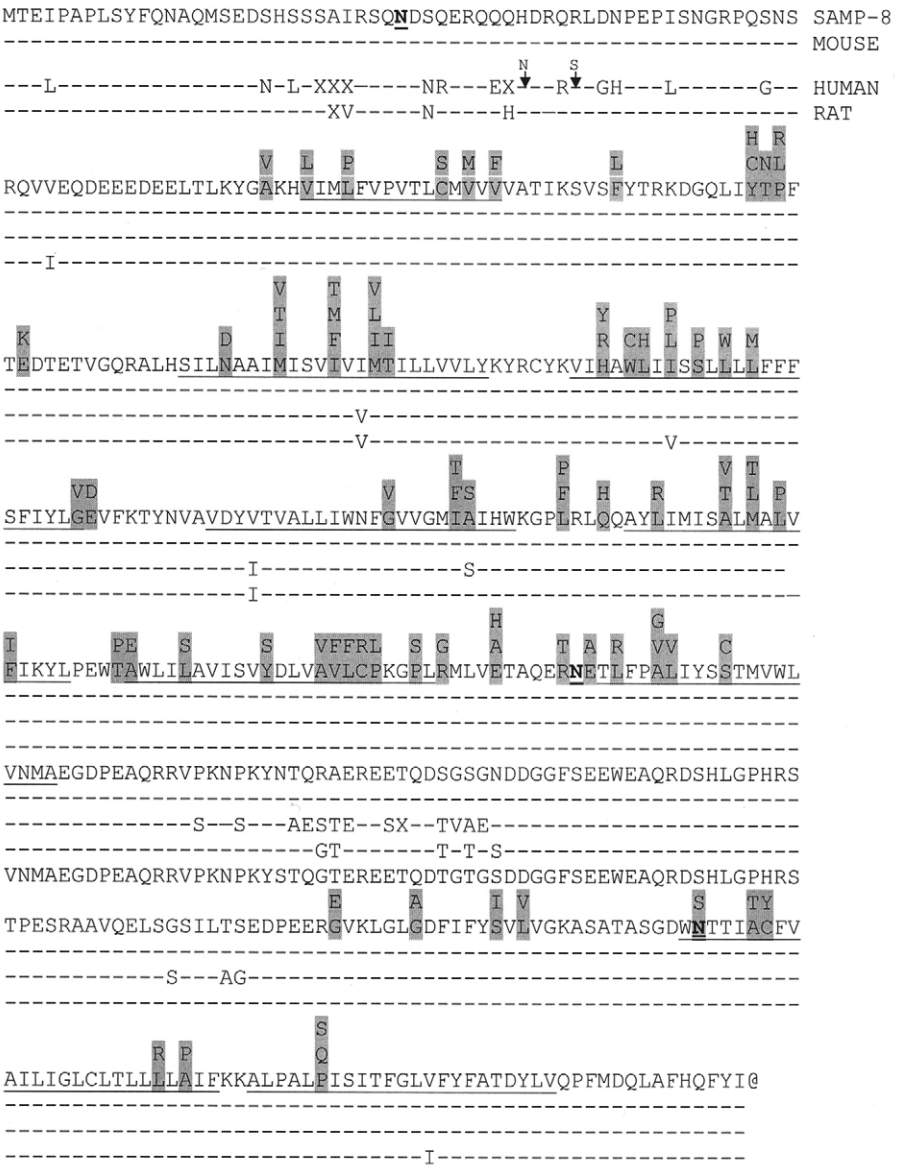

Fig. 1. Comparison of the amino acid sequence of PS1 of SAMP8 with that of house mouse, human and rat. Shaded regions represent the mutations linked to familial Alzheimer's disease (FAD) in human sequences reported thus far. Common sequences are represented by hyphens. Glycosylation sites are represented by bold letters, and transmembrane regions are underlined. Arrows show the substitution of specified amino acids.

Sequencing

The clones were subjected to double-stranded sequencing as described previously (Kumar, 1993). The amino acid sequence was deduced from the coding frame using the Gene Runner program (http://www.generunner.com).

Western blotting

Western blotting was performed essentially as described previously (Kumar et al., 2001). Briefly, 10–20 mg of hippocampal tissue from 4-month-old mice was homogenized in PBS containing protease inhibitors (1% Triton-X100, 2 mmol l^{-1} PMSF, 3 mmol l^{-1} EDTA and 0.1% SDS). Protein (2–5 μg) was run on 10% tris-glycine polyacrylamide gels and transferred to nitrocellulose nylon membranes. The blot was blocked with 1% milk protein in TBS (25 mmol l^{-1} Tris-HCl, pH 7.4, 137 mmol l^{-1} NaCl, 2.7 mmol l^{-1} KCl) followed by treatment with the first antibody for 1 h. The antigen–antibody complex was detected by using the second antibody conjugated to peroxidase, followed by ECL (advanced Western Blotting Detection kit, GE Healthcare, Buckinghamshire, UK) luminal reaction and exposure for a few seconds to X-ray film.

RESULTS

Fig. 1 shows a comparison of the amino acid sequence of PS1 from the SAMP8 mice with those of the house mouse, human and rat sequences. A comparison of the SAMP8 sequence with that published for mouse revealed that the SAMP8 PS1 did not exhibit any differences either at the amino acid level or at the nucleotide level. However, a difference in amino acid sequences of 6.6% and 3.4% was observed with the human and rat sequences (Taniguchi et al., 1997), respectively. Considering the minor changes in the sequence among these species, PS1 is structurally and evolutionarily conserved. The possible nine transmembrane domains (underlined), putative glycosylation sites (marked by bold letters) and the changes in amino acid sequence between species are shown in Fig. 1. Shaded regions in the figure represent the reported mutations observed in FAD patients. In addition, PS1 also exhibits two hydrophilic acidic domains that are not altered between the species.

We further investigated hippocampal PS1 for any possible quantitative changes in expression with aging. This analysis was performed by western blotting. For this, similar amounts of protein, as estimated by the Bradford colorimetric assay (Bradford, 1976), were separated on 4–12% bis-tris gels with MES buffer, transferred to PVDF membranes and probed with anti C-terminal-specific antibodies. Anti PS1 antibodies were used for the detection of the PS1 55 kDa holoprotein and the 150 kDa heterodimer (Fig. 2A).

The densities of the PS1 bands were normalized to the densities of GAPDH bands obtained using the same quantities of protein loaded on identical gels, blotted and probed with GAPDH antibodies or by stripping the same blot (Fig. 2B).

Fig. 3 shows the plot of ratios of band intensities of P8 PS1 and the corresponding GAPDH bands shown in Fig. 2. The results show that the amount of holoprotein increased from 4 to 18 months (Fig. 3A). Similar quantification of the heterodimer showed a pronounced decrease with age (Fig. 3B). Fig. 3 represents an average of three animals per experiment.

Fig. 4 shows the western blotting of the hippocampal extracts from three age groups of CD-1. The amounts of protein loaded and the blotting protocol are identical to those used with the SAMP8 hippocampal extracts. In this experiment, we did not observe any change in the density of protein bands.

Fig. 5 shows the ratio of the intensities of the CD-1 PS1 and corresponding GAPDH bands shown in Fig. 4. PS1 of the control CD-1 only showed a small decrease with age. The amount of heterodimer also exhibited a decrease with age (Fig. 5B).

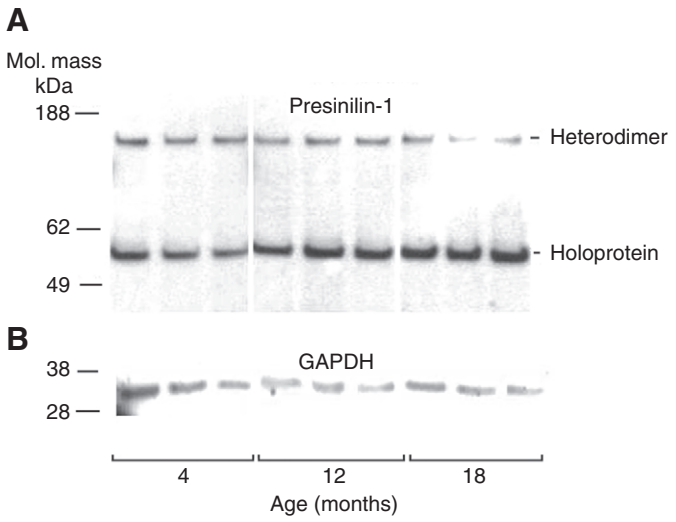

Fig. 2. Western blots of hippocampal PS1 from various age groups of SAMP8 mice. The hippocampal tissue was homogenized and subjected to western blotting as described in the text. PS1 was detected using anti-PS1 polyclonal antibody (A). The same blot was stripped for 10 min and re-probed with GAPDH monoclonal antibody (B).

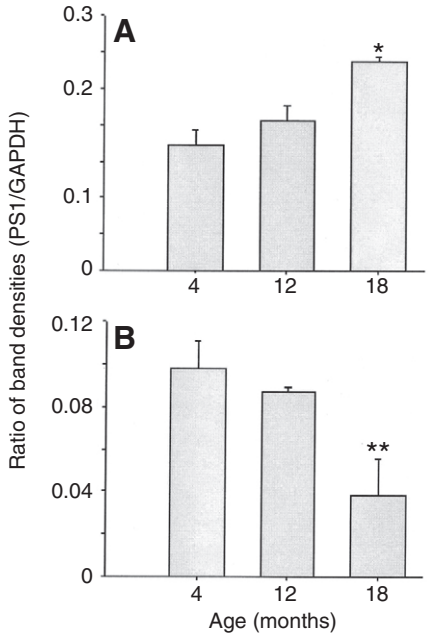

Fig. 3. Quantification of hippocampal PS1 of different age groups of SAMP8 mice: (A) holoprotein; (B) heterodimer. The bands obtained in Fig. 2 were scanned and quantified using UnScan it program (Silk Scientific, Orem, UT, USA). The total density obtained in each band is expressed as the number of pixels. The ratio of densities of PS1 and GAPDH bands is plotted against the age of the mouse. *P*-value by one-way ANOVA between age groups is <0.02 (*) and <0.031 (**). The change in holoprotein level normalized to GAPDH is 0.17±0.02 to 0.28±006.

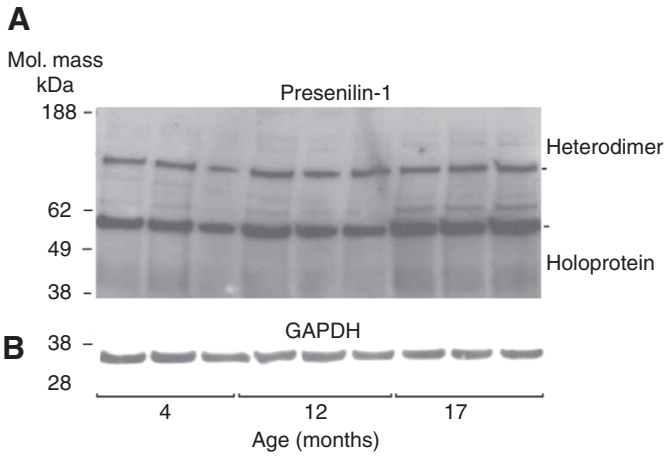

Fig. 4. Western blotting of hippocampal PS1 from various age groups of CD-1 mice. The hippocampal tissue was homogenized and subjected to western blotting as described in the text. PS1 was detected using anti-PS1 polyclonal antibody (A). The same blot was stripped for 10 min and re-probed with GAPDH monoclonal antibody (B).

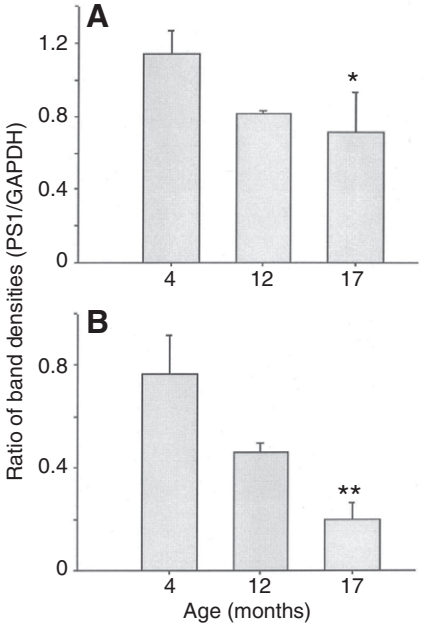

Fig. 5. Quantification of hippocampal PS1 from various age groups of CD-1 mice: (A) holoprotein; (B) heterodimer. The bands obtained in Fig. 4 were scanned and quantified as in Fig. 3. The total density obtained in each band is expressed as the number of pixels. The ratio of densities of PS1 and GAPDH bands is plotted against the age of the mouse. *P*, determined by one-way ANOVA, is <0.17 (*) and <0.013 (**). The change in the holoprotein levels normalized to GAPDH is 1.14±0.12 to 0.71±0.21.

DISCUSSION

As PS1 is an integral protein of the γ-secretase complex, its stoichiometry may play an important role in balanced processing of APP. A decrease in PS1 (Refolo et al., 1999) or inactivation by mutations (Sudoh et al., 1998) may be associated with increased $A\beta_{42}$. We had previously noticed an increase in APP as well as $A\beta_{1\text{-}42}$ with age in SAMP8 mice (Morley et al., 2000), suggesting that a decrease in PS1 may cause increased aberrant γ-secretase activity. Accumulation of Aβ is attributed to loss of memory, as reduction of APP expression reverses this loss (Kumar et al., 2000). Therefore, reduction in APP expression is one of the pharmaceutical approaches to counter age-dependent or neurodegenerative disorders like AD that involve memory loss. Being an essential component of γ-secretase, PS1 is another therapeutic target for reducing Aβ formation. As SAMP8 mice have been shown to exhibit memory loss at a relatively early age (Flood and Morley, 1998; Miyamoto, 1997), we have studied the changes in PS1 expression with age in these mice. Using *Xenopus* oocytes, Heyn and Vulliet have shown that APP expression is more pronounced with mutated PS1 (Heyn and Vulliet, 2001). Therefore, it is conceivable that PS1 regulates the expression of APP consequent to the formation of Aβ, by a feedback mechanism (if more APP is processed, more of it will be expressed). In the current investigation, we suggest that γ-secretase activity may become less regulated by the increased expression or mutation of PS1, leading to an increased Aβ, which may in turn cause early loss of memory in SAMP8 mice.

Several reports suggest that inhibition of the expression of APP or secretases would reduce Aβ accumulation (McMahon et al., 2001; Nawrot, 2004; Tomita and Iwatsubo, 2004). A few potent inhibitors of β- and γ-secretases have been reported *inter alia* (Dominguez et al., 2001; Maiorini et al., 2002; Schenk et al., 2001; Shearman et al., 2000; Tomita and Iwatsubo, 2004), which is rational in view of the fact that, even recently, β-secretase has been shown to be elevated in AD patients (Zhao et al., 2007). These inhibitors are small-molecular-mass chemicals that inhibit secretase activity. γ-secretase is a high-molecular-mass membrane protein complex that includes PS1, nicastrin, anterior pharynx homolog 1 (APH-1) and presenilin enhancer protein 2 (Pen-2) (Morohashi et al., 2006). It was shown that highly specific inhibitors that modulate PS1 activity in human CNS neurons not only affected Aβ generation but also affected Notch and its activity (Seiffert et al., 2000). This was accompanied by changes in neurite morphology, suggesting that regulation of γ-secretase/PS1 activity may have clinically beneficial effects on the neuritic pathology of AD (Figueroa et al., 2002). Antisense oligonucleotides (Dolnick, 1991), RNA cleaving agents like ribozyme (Macpherson et al., 1999) and RNA interference (Elbashir et al., 2001) have recently taken strides as therapeutic agents that regulate messages thereby regulating the corresponding protein expression. Targeting γ-secretase activity is one of the therapeutic approaches for AD (Seiffert et al., 2000; Tomita and Iwatsubo, 2004). Reduction of PS1 was shown to downregulate amyloid (Luo et al., 2004; Saura et al., 2005). We have observed that inhibition of PS1 in SAMP8 mice improves memory (B.V.K., S.A.F., W.A.B.,M.F. and J.-E.M., unpublished). Downregulation of PS1 was shown to decrease the secretion of amyloid β protein (Luo et al., 2004). Refolo et al. noticed that antisense against PS1 increased the secretion of $A\beta_{42}$ without affecting $A\beta_{40}$ in transfected human cell cultures (Refolo et al., 1999). It is possible that either the increase in PS1 or its inactivation by mutation may result in the aberrant activity of γ-secretase, consequently increasing the production of Aβ protein. Our group has shown that the efflux of amyloid β protein in SAMP8 mice is impaired (Banks et al., 2003). Therefore, it is also possible that PS1 may assist in the removal of excess amyloid β protein. Interestingly, while an excess amount of amyloid β protein causes impairment of memory, too little of it may also be detrimental to cognitive functions. We have recently shown that blocking amyloid β protein with a specific peptide antibody resulted in impaired acquisition in CD-1 mice (Morley et al., 2005). Taking these results together, PS1 may be involved in the regulation of

$A\beta_{42}$ secretion in addition to aiding γ-secretase activity. Most importantly, it may serve as a regulator of γ-secretase activity. If this were the case, the therapeutic approach should involve coordinated reduction of γ-secretase and PS1 rather than just one of the components of this enzyme complex. Regardless of the overall function of PS1, upregulation or downregulation of PS1, depending on the available PS1, may be one of the therapeutic approaches in AD pathogenesis. We are currently in the process of developing novel technologies to upregulate or downregulate proteins. In a separate study we have shown that expression of PS1and APP may be regulated by hybrid antisense technology in transiently co-transfected COS 7 (B.V.K., M.F. and P.K., unpublished). Such molecular modulation of more than one protein at a time may become necessary to offset the adverse affects of simultaneous over- and/or under-expression of certain important proteins.

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