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A Correspondence between Aging-related Reduction of Neprilysin and Elevation of Aβ-42 or γ-Secretase Activity in Transgenic Mice Expressing NSE-controlled APPsw or Human Mutant Presenilin-2

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Abstract – Neprilysin (Nep) is known to be important to degrade Aβ derived from amyloid precursor protein (APP) by cleavage with β-and γ- secretases. In order to determine whether a correspondence between Aβ-42/γ-secretase activity and Nep levels exists in postnatal aging of transgenic mice expressing either neuron-specific enolase (NSE)-controlled human mutant presenilin-2 (hPS2m) or APPsw alone, the levels of Nep expression and Aβ-42/γ-secretase activity were examined at age of 5, 12, and 20 months, respectively. The levels of Nep expression in both types of transgenic brains were decreased relative to those of control mice in a aging-related manner, while the level of Aβ-42/γ-secretase activity was reversibly increased. Thus, changes in Aβ-42 may all reflect variation in amounts of Nep enzyme.

Key words □ Alzheimer, neprilysin, amyloid, transgenic.

INTRODUCTION

Alzheimer's Disease (AD), the most common cause of dementia in elderly humans, occurs when neurons in the memory and cognition regions of the brain are accompanied by massive accumulation of abnormal fibrous amyloid β-protein (Aβ) that is deposited as extracellular senile plaques, composed of the 39 to 43 amino-acid long peptides derived from the amyloid precursor protein (APP) by cleavage with β- and g-secretase. Mutations in AD genes cause an increase in the anabolism of Aβ-42, leading to Aβ deposition and accelerating AD pathology. Thus, reducing Aβ production in the brains or the activation of mechanisms that accelerate its clearance from brains has become major targets for the development of drugs, since the metabolic balance between Aβ anabolic and catabolic activities might be responsible for the Alzheimer's disease (Glabe et al, 2000).

Proteolytic enzymes to degrade Aβ are important to maintain and regulate Aβ level. Its enzymes include neprilysin (Nep), insulin-degrading enzyme (IDE), and endotherin-converting enzyme (ECE). Of these enzymes, only neprilysin has been identified as a major Aβ degrading enzyme in the brain (Iwata et al, 2000). Nep known as neutral endopeptidase (EC 3.4.24.11) is a 90 to 110 kDa type II membrane-bound zinc-metallopeptidase that is composed of a short N-terminal cytoplasmic membrane spanning region and a large C-terminal extracellular, catalytic domain containing HExxH Zn-binding motif (Turner, 2001). Nep hydrolyzes extracellular oligopeptide (5kDa) on the amino side of hydrophobic residues, and this oligopeptide is a candidate for degradation of the hydrophobic Aβ40- and 42-peptides. Nep was also identified as the enzyme possessing Aβ-degrading activity in brain tissue (Iwata, 2000), and its transcript level was reduced in amyloid-burden areas of sporadic AD brain tissue (Yasojima et al, 2001a; Yasojima et al, 2001b). Thus, amyloid formation may be caused by either increased production or degradation of Aβ-42

In normal mice, levels of Nep expression were selectively decreased at the outer molecular layer of the dentate gyrus, and the stratum lucidum, and the terminal zones of lateral perforant path (Iwata et al, 2002), which are highly vulnerable region of AD pathology (Gomez-Isla et al, 1996). Age-related decline of NEP activity in these specific regions in normal mice is likely

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to promote the Aβ catabolism in brain tissue from Alzheimer's diseased-transgenic mice.

Transgenic line, expressing either NSE-controlled APPsw or human mutant presenilin-2 (hPS2m) alone has been produced by us previously (Hwang et al, 2002; Hwang et al, 2004). These transgenic mice showed an elevated level of Aβ-42 at the 12 months of age in their brains. The aims of this study were to determine whether the expression of Nep is decreased during the postnatal aging at ages of 5, 12, and 20 months, and if this is correlated with elevated levels of Aβ-42/γ-secretase activity in the transgenic brains.

MATERIALS AND METHODS

Animals

Transgenic and non-transgenic littermates used in these experiments were handled in an accredited Korea Food and Drug Administration (FDA) animal facility in accordance with the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC) International Animal Care Policies (Accredited Unit-Korea Food and Drug Administration: Unit Number-000936), and maintained in a specified pathogen-free state. All the mice were housed in cages under a strict light cycle (lights on at 08:00 h and off at 20:00 h), and given a standard irradiated chow diet (Purina Mills Inc.) *ad libitum*.

Western blot analysis

The brain tissues were homogenized with 1% Nonidet P-40 in 150 mM NaCl, 10 mM Tris HCl (pH 7.5), and 1 mM EDTA supplemented with protein inhibitor mixture (Roche) followed by centrifugation at 10,000xg for 10 min at 4°C. The cell lysates run on 10% polyacrylamide gel, transferred to nitrocellulose membranes, and membrane was then incubated with primary anti-βAPP (anti-βAPP, Zymed) and anti-Nep (Santa Cruz) antibodies to detect Aβ-42 and Nep expressions. Each complex of antigen-antibody was visualized with biotylated secondary antibody (goat anti-rabbit)-conjugated HRP streptavidin (Zymed, Histostain-Plus Kit). Expression of β-actin was analyzed by western blot for a control.

γ-Secretase assay

The brain tissues were homogenized, resuspended in 3 volumes of buffer A (10 mM Tris-HCl (pH7.4), 1mM EDTA, 250 mM sucrose, and PMSF). The lysate was then fractionized by differential centrifugation at 900xg and 4°C, for 10 min, followed by 110,000xg for 75 min. The final pellet containing the intracellular proteins was washed, resuspended in Buffer A, with 1% Triton X-100, and stored at -80°C. The intracellular proteins were then tested for secretase activity by the addition of a secretase-specific peptide conjugated to the reporter molecules EDANS and DABCYL (γ-Secretase Activity Kit, R&D System, Inc.). Cleavage of the peptide by the secretase physically separates the EDANS and DABCYL allowing for the release of a fluorescent signal. The level of secretase activity in the cell lysate is proportional to the fluorometric reaction.

Statistical analysis

Tests for significance were performed using One-Way analysis variance (SPSS for Window, Release 10.01, Standard Version, Chicago, IL). All values are reported as the mean ± standard deviation. Statistical significance was set at $p<0.05$.

RESULTS

Correspondence between Nep reduction and Aβ-42 elevation during postnatal ages of transgenic brains

To determine if there might be a correspondence between Nep reduction and Aβ-42 elevation during postnatal ages of transgenic brains, western blot was performed in the brains of transgenic mice at ages of 5, 12, and 20 months, and result was compared to those of non-transgenic normal mice. The level of Nep protein in both types of transgenic brains were decreased relative to those of control mice in an ages-dependent manner, while the levels of Aβ-42 gradually increased (Fig. 1).

γ-Secretase activity was increased in an age-dependent manner

A correspondence was found between the reduction in the Nep and induction in the Aβ-42 in an age-dependent manner (Fig. 1). It raised a possibility that γ-secretase may be more activated than the α-secretase in the brains of both transgenic mice. To test this, lysate was prepared from the brains of transgenic or non-transgenic mice, and γ-secretase activity was measured. The levels of γ-secretase activity in the brains of transgenic mice were higher than those in their non-transgenic mice (Fig. 2).

DISCUSSION

There are several implications in the understanding of the correlation of Nep with an Aβ-42 or a γ-secretase activity during the postnatal development. One primary conclusion is that a

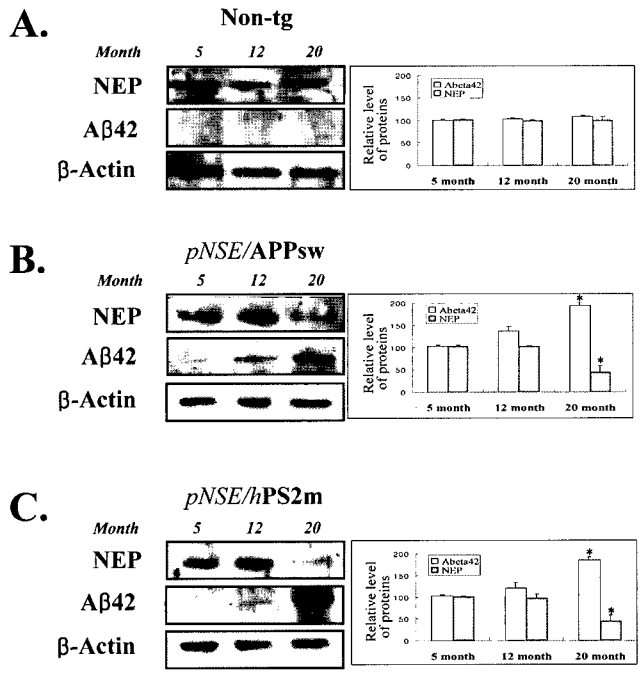

Fig. 1. Age-related changes in Nep and Aβ-42 levels in transgenic and non-transgenic mice. Lysates were prepared from the brains at indicated age. Proteins were separated by 10 %SDS-PAGE and transferred to nitrocellulose membrane. Nep and Aβ-42 detected with antibodies raised against human Nep and Aβ-42 followed by anti-rabbit-HRP visual. Protein levels in each age of development were quantified by a Kodak Electrophoresis Documentation and analysis system. Three mice per age group were assayed in triplicate on western blot analysis. Median value and SD are shown. *P<0.05 vs 5 and 12 months age of mice.

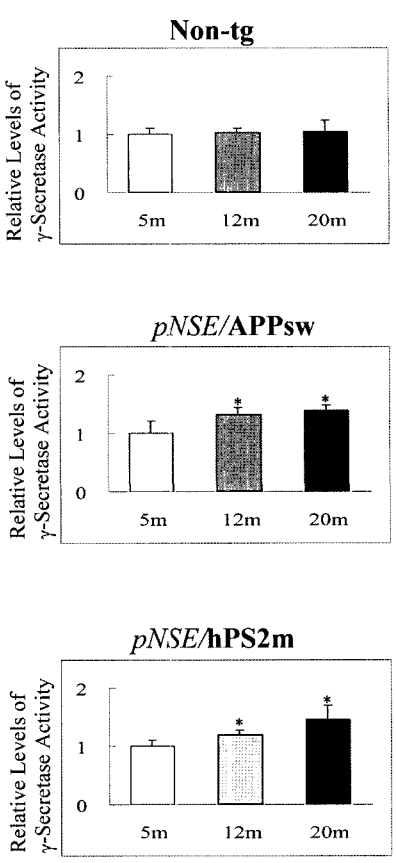

Fig. 2. γ-Secretase activity in transgenic and non-transgenic mice. Identical lysates prepared from the brains at indicated age were used for assaying γ-secretase activity in triplicate and quantified by a Kodak Electrophoresis Documentation and analysis system. Three mice per age group were assayed in triplicate on western blot analysis. Median value and SD of three mice are shown. *P<0.05 vs 5 and 12 -months age of mice.

reduction in the levels of Nep was observed in the both types of old mutant transgenic brains. This finding was not consistent with a report that the levels of cortical Nep protein did not differ between transgenic and non-transgenic littermates regardless of postnatal ages, although cortical Nep mRNA levels in 22-months-old Tg2576-transgenic mice were significantly lower relative to 2-months-old (Apelt et al, 2003). Since, Nep expression in the brains is regulated in a cell-specific manner (Lu et al, 1995; Lu et al, 1996), it is possible that cerebral cortices is not area where is not abundant Nep proteins. Noticeably, a clear change in Nep protein level with aging observed in the hippocampal formation, in which the level was reduced by 20% at 132 weeks, compared to the 10-weeks group of mice (Iwata et al, 2002). The second conclusion is that the age-related enhancements of both levels of Aβ-42 and g-secretase activity were observed in the brains of hPS2m- and APPsw-transgenic mice. These results are consistent with the results that the levels of Aβ-42 were increased in the brains of 12-months old of transgenic mice expressing either hPS2m or APPsw alone (Hwang et al, 2002, Hwang et al, 2004). It is possible that significant induction in the levels of Aβ-42 is the result of the elevated levels of γ-secretase activity. The third conclusion is that the levels of Nep expression was low at 20 months ages of transgenic mice, but Aβ-42 level was high at 20-month-age of transgenic brains. Obviously, there is a coincidence between Nep reduction and Aβ-42 or g-secretase activity elevation. It is similar to a report that Nep protein as well as mRNA levels have been found to be down regulated in Alzheimer's disease, (Yasojima et al, 2001a; Yasojima et al, 2001b; Akiyama et al, 2001). This finding is likely to be due to a failure in β-amyloid degradation by Nep expression that might not catalyze directly Aβ-42 peptides. However, injections of syn-

thetic b-amyloid peptide in transgenic Tg2576 mice led to an increase in the levels of Nep and a reduction in the levels of β-amyloid (Mohajeri et al, 2002). Although, it is hard to illustrating this mechanism, a lack of feedback of increasing amounts of the injected Aβ-42 substrate on the expression level of Nep.

In conclusion, the paper provided the experimental evidence that the level of Nep expression in both types of transgenic brains were decreased relative those of control mice in a aging-related manner, while the level of Aβ-42/γ-secretase activity was reversibly increased.

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