SAGE KE SCIENCE OF AGING KNOWLEDGE ENVIRONMENT

Alzheimer's Disease, Neuropeptides, Neuropeptidase, and Amyloid-β Peptide Metabolism

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Amyloid β peptide (Aβ), the pathogenic agent of Alzheimer's disease (AD), is a physiological metabolite in the brain. We have focused our attention and effort on elucidation of the unresolved aspect of Aβ metabolism, proteolytic degradation. Among a number of Aβ-degrading enzyme candidates, we used a novel *in vivo* paradigm to identify a member of the neutral endopeptidase family, neprilysin, as the major Aβ catabolic enzyme. Neprilysin deficiency results in defects in the metabolism of endogenous Aβ40 and 42 in a gene dose-dependent manner. Our observations suggest that even partial down-regulation of neprilysin activity, which could be caused by aging, can contribute to AD development by promoting Aβ accumulation. Moreover, we discuss the fact that an aging-dependent decline of neprilysin activity, which leads to elevation of Aβ concentrations in the brain, is a natural process that precedes AD pathology. In this Perspective, we hypothesize that neprilysin down-regulation has a role in sporadic AD (SAD) pathogenesis and propose that this knowledge be used for developing novel preventive and therapeutic strategies through utilization of a G protein-coupled receptor (GPCR).

Keywords. mammalian aging, mutations, aggregates, cell senescence, cell loss, life extension, Alzheimer's disease

If humans lived to be 120 to 140 years of age or more, there is a serious possibility that all would develop Alzheimer's disease (AD) (see "**Detangling Alzheimer's Disease**" http://sageke.sciencemag.org/cgi/content/full/sageke;2001/1/oa2) in the later years of the human life-span. This prediction stems from the fact that the incidence of AD increases exponentially after the 7th decade of life, although it is not certain that this trend continues in persons beyond age 100. Thus, epidemiological studies indicate that one out of two people who reach the age of 85 and nine out of 10 people who attain the age of 100 will be affected by AD or AD type brain pathology (*1*) (see "**Honig Case Study**" http://sageke.sciencemag.org/cgi/content/full/sageke;2001/1/dn2). What is the significance of this fact? The answers are obviously not entirely clear, as so few individuals have been studied who have attained these ages. But one interpretation is that AD or AD type pathology may be an ultimate form of brain aging. Note that here we mean to imply that "brain aging" differs from "general aging" in that mitotic quality control mechanisms play relatively minor roles simply because differentiated neurons in the adult brains are primarily post-mitotic cells.

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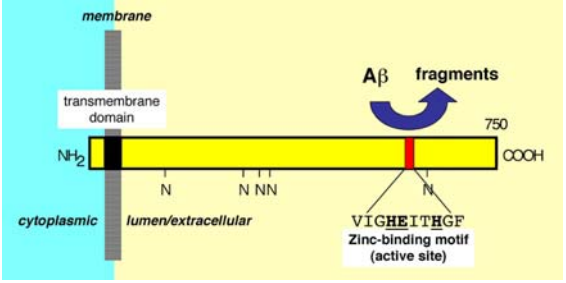
Until several centuries ago, most human beings lived only 50 to 60 years, because of harsh living conditions that led to high infant and early adult mortality. Infectious diseases were rampant, and no effective therapies existed; thus large numbers of children and adults succumbed to bacterial infections. During this time in history, there was practi-cally no "brain aging" of the sort that we have been studying during the last few decades. Therefore, the time period between the ages of 50-60 to 80-100 years for human beings signals a very new biological time period in the course of the evolution of life, which has taken hundreds of million years. Thus, in our view, understanding the mechanism of the aging-dependent develop-ment of AD is, at least in part, equal to understanding the new biological time period, which did not even exist until recently.

This reasoning intimates that some aspects of the biological mechanisms originally designed to be beneficial in maintaining metabolic homoeostasis through negative feedback processes when humans lived to be 50 to 60 years of age could become harmful after such ages have been exceeded. Therefore, an under-standing of the mechan-isms of brain aging, the ultimate form of which may well be re-presented by AD, should help scientists to create novel strate-gies for controlling some aspects of what has, until now, been con-sidered to be normal aging.

In this Perspective, we describe our current experimental evidence-based hypo-thesis that might associate the metabolic homeostasis of neuropeptide(s) in the brain with the aging-dependent development of sporadic AD (SAD) through alteration in the metabolism of amyloid β peptide (Aβ). It was established during the 1990's, a decade of phenomenal progress in understanding familial AD (FAD), that Aβ [which is derived from amyloid precursor protein (**APP** http://sageke.sciencemag.org/cgi/genedata/sagekeGdbGene;197) via the action of β- and γ-secretase] acts as a potent pathogenic agent in the development of AD. Such conclusions were drawn as a consequence of the identi-fication of FAD-causing gene mutations and analyses of their molecular and pathological phenotypes (*2*). We consider the micro-tubule-associated protein tau to be as import-ant as Aβ, particularly in clinical terms, now that mutations in the tau gene have been shown to cause tauopathy and neurodege-neration (*3*), but this topic is beyond the scope of this Perspective. We also propose here a new approach to the metabolic control of Aβ concentrations in the brain via the modulation of neuropeptide-receptor systems, for the prevention and treatment of AD. Note that all of the neuropeptide receptors are G protein-coupled receptors (GPCRs), which are the most tractable and relevant pharma-ceutical targets in molecular pharmacological terms (*4*).

Neuropeptides and Alzheimer's Disease

In 1980, Davies and colleagues (*5*) dis-covered that concentrations of the neuro-peptide somatostatin were significantly re-duced in the brains of AD patients. This observation has been



Animation 1. Schematized structure of the major Aβ-degrading enzyme, neprilysin.
Neprilysin is a type II membrane-associated peptidase with its active site facing the lumen or extracellular side of plasma membranes. This topology is favorable for the degradation of extracytoplasmic peptides such as Aβ. Presynaptic terminals and nearby intracellular (lumen-sided) locations are likely to be the sites of Aβ degrada-tion by neprilysin.

confirmed repeatedly by others, but its pathogenic significance has never been fully resolved. Because concen-trations of other neuropeptides, such as vaso-pressin, neuropeptide Y, substance P, and corticotropin-releasing factor (CRF), have also been reported to be reduced in AD brains (*6-12*), the general interpretation has been that the reduction is likely to be, at least in part, a onsequence of the degeneration of neuropeptide-generating neurons in AD brains. This assumption does not, however, exclude the possibility that reduction of a specific neuropeptide(s) may be causal in the pathological cascade of AD development. Consistent with this alternative hypothesis is the finding that the amounts of some neuropeptides decline upon aging before the onset of AD (*13,14*).

Fig. 1. Current list of Aβ-degrading enzyme candidates studied using reverse genetic techniques.

KO or KI mice	Aβ42 (Primarily pathogenic)	Aβ40 (Secondarily pathogenic)
Neprilysin-KO(-/-)*[1]	2-fold increase	2-fold increase
Neprilysin-KO(+/-)*[1]	1.5-fold increase	1.5-fold increase
ECE 2-KO (-/-)*[2]	1.2-fold increase	1.3-fold increase
ECE 2-KO (+/-)*[2]	1.15-fold increase	1.2-fold increase
ECE 1-KO (+/-)*[2]	1.2-fold increase	1.2-fold increase
IDE-KO (-/-)*[3]	No significance*	1.1-1.2-fold increase
tPA-KO (-/-)*[4]	No significance*	No significance*
uPA-KO (-/-)*[5]	No significance*	No significance*
ACE-KO (+/-)*[6]	No significance*	No significance*
FAD presenilin 1-KI*[7] **(positive control)**	typically 1.5-fold increase	No significance*

KO: (gene) knock-out; KI: (gene) knock-in; ECE: endothelin-converting enzyme; IDE: insulin-degrading enzyme; tPA: tissue-type plasminogen activator; uPA: urokinase; ACE: angiotensin-converting enzyme. *No significance if the difference compared to control mice is <10%. *1: see (*18*); *2: see (*25*); *3: see (*58*); *4: see (*59*); *5: see (*60*); *6: see (*61*); *7: see, for instance, (*3*). The results with mutant presenilin 1-KI mice represent typical pathogenic alterations in the Aβ levels, as a positive control, leading to accelerated Aβ accumulation in the brain. The quantification was performed using an identical ELISA developed by Nobuhiro Suzuki (*62*). The mice were ~8 to 10 weeks of age. Gender is likely to be male in each study. T.C.S. accepts the responsibility if any of the data are in error.

Meanwhile, our laboratory has been en-gaged in an effort to identify the physiologi-cally relevant peptidase responsible for the in vivo degradation of Aβ in the brain (*15-18*); as described in the next section, the prime suspect, neprilysin, (see **Animation 1** http://sageke.sciencemag.org/cgi/content/full/sageke;2003/3/pe1/DC1) is not only involved in the metabolism of neuropeptide(s), but also is likely to be regulated by neuropeptide(s) as part of the homeostatic mechanisms present in the brain.

Accordingly, what Davies and others discovered in the 1980's (*5*) and what we identified during the past few years as the major in vivo Aβ-degrading enzyme (*18*) now may be combined to form-ulate a plausible strategy to eluci-date the most fun-damental question in AD research: What causes the aging-dependent accumu-lation of Aβ, □□□□□ leads to the development of SAD? Moreover, thoughtful integration of these seemingly disparate findings may lead to the development of a new therapy for AD. Such a therapy would not be expected to have the types of side effects that are anticipated for inhibitors of β- and γ-secretase, which may also inhibit the biologically indispensable processing of APP http://sageke.sciencemag.org/cgi/genedata/sagekeGdbGene;197 and other important substrates (*19-21*).

Metabolism of Aβ: The Current Status of Research on the Aβ-degrading Enzymes

Because Aβ is constantly anabolized and catabolized in the brain, the steady-state concentrations of Aβ are determined by the dynamic balance between anabolic and catabolic activities (*22,23*). In contrast to most cases of dominantly inherited FAD and mouse models of Aβ amyloidosis, elevation of Aβ anabolism in the brain prior to the occurrence of the Aβ pathology is rarely observed in normal aging. One logical presumption, then, is that Aβ deposition in a large number of SAD cases might be caused primarily by its reduced catabolism [see (*23*) for further details].

During the past few years, several groups have proposed a variety of candidates for the "real," or authentic Aβ-degrading enzymes [see (*22,24,25*) for reviews]. Fig. 1 describes the current list of seemingly relevant

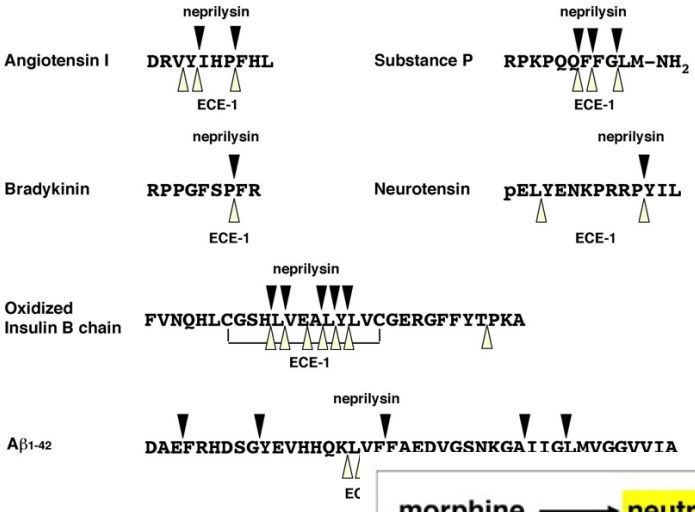
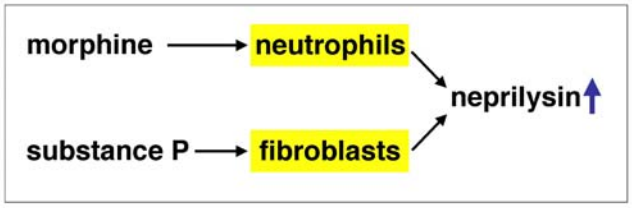


Fig. 2. Cleavage sites of sever pro-teolysis catalyzed by neprily enzyme-1 (ECE-1). Neprilysin and E M13 clan of peptidases, share simi respect to their substrates. See (*15, 2*



The receptors are likely to be GPCRs.

Animation 2. Regulation of neprilysin activity/expression by ligand-receptor mechanisms. Morphine and substance P have been shown to increase neprilysin activity/expression in neutrophils and fibroblasts, respectively. All of the receptors for such peptide ligands identified thus far are GPCRs.

can-didates studied using reverse genetics tech-niques. Among these candidates, nepri-lysin and endothelin-converting enzymes (ECEs), which both belong to the M13 clan of metallopeptidases, appear to account for 60 to 80% of the total Aβ-degrading activity in brain tissue [see (*24*) for the mathematical basis of this assumption]. Because of the differences in their enzymatic and cellular properties, neprilysin and ECEs are likely to play complementary roles in distinct subcell-ular compartments; the former degrades Aβ inside secretory vesicles and on the extra-cellular surface, while the latter does so in acidic compartments represented by the trans-Golgi network (*23-25*). Incidentally, these peptidases share some neuropeptides as common substrates (*15,25,26*) and thus could be grouped on this basis into a particular family of neuropeptidases (see Fig. 2).

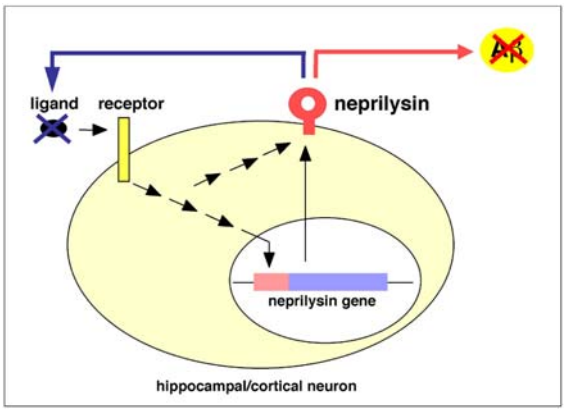
In any case, at the present time, nepri-lysin (see **Animation 1** http://sageke.science mag.org/cgi/content/full/sageke;2003/3/pe1/DC1) is the most potent Aβ-degrading enzyme identified in vivo. It is noteworthy that even a heterozygous deficiency of nepri-lysin leads to an approximately 1.5-fold increase in Aβ concentrations in brain tissues, a phenotype comparable to those of the FAD-causing gene mutations. Assuming that the parallel with FAD is relevant [see Figure 1.8 of (*24*)], this finding suggests that if neprilysin activity is reduced in human brains to ~50% at the age of 50, the affected individuals will develop AD in 30 to 60 years after this time. Consistent with this hypo-thesis, the McGeer group has demonstrated that, compared to control mRNAs, neprilysin mRNA concentrations in SAD patients, at relatively early stages of the disease process (Braak Stage II), are significantly and select-ively reduced in the hippocampus (a part of the limbic system located in the medial tem-poral lobe and important in memory forma-tion), the midtemporal gyrus (a convolution or ridge on the surface of the cerebrum), and, to a lesser extent, the cerebellum (the part of the brain that lies just below the posterior part of the cerebrum and plays an essential role in coordinating voluntary movement, controlling muscle tone, and maintaining balance). The fact that the amounts of control neuronal mRNAs that encode cyclophilin and microtubule-associated protein 2 (MAP2) were not changed in tissues from SAD patients indicates that down-regulation of neprilysin expression is not simply a con-sequence of neurodegeneration (*27, 28*).

Neprilysin, Neuropeptides, and Aging

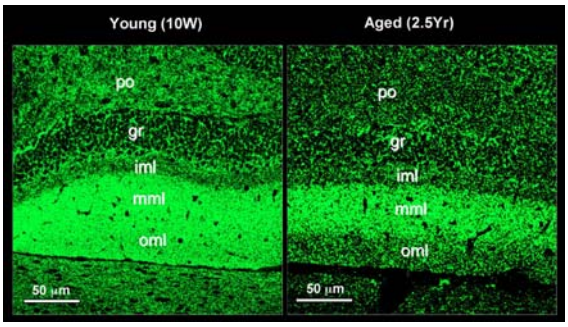
Enkephalin is a 5-amino acid neuropep-tide that is primarily involved in analgesia (pain regulation). Neprilysin was originally identified biochemically as an enkephalin-degrading enzyme in a test tube-based ex-perimental paradigm and was once termed "enkephalinase" (*29-31*). However, because enkphalin concentrations remain essentially unchanged in the brains of neprilysin-knock-out (KO) mice (*32*), neprilysin does not appear to be the major rate-limiting enkepha-lin-degrading enzyme in the brain as a whole. These observations illuminate the danger of depending too much on in vitro assays to predict the in vivo functions of a given mole-cule, an important caveat in AD research. On the basis of a collection of in vivo and in vitro experiments, we predict that relatively small peptides that can be degraded by exo-peptidases--such as aminopeptidases, dipep-tidyl peptidases, tripeptidyl peptidases, carboxy peptidases, and peptidyl dipepti-dases—probably do not require neprilysin activity for their cata-bolism as long as they are acce-ssible to these peptidases. Becau-se neprilysin is incapable of de-grading peptides larger than ~5 kDa (~50 amino acids) (*29-31*), peptides composed of ~10 to 40 amino acid residues are likely to be relevant substrates. However, the secondary and tertiary struc-tures of the substrates also need to be taken into account, because the 3-D structure of neprilysin indicates that only substrates that fit into the active site of the en-zyme can be proteolyzed effici-ently (*33*). Indeed, an opioid pep-tide 5 amino acids in length was shown to be elevated in a limited region of the hippocampal forma-tion of neprilysin-KO mice (*34*), contrary to the results of the bulk biochemical quantification (*32*). This finding indicates that the relative function of neprilysin de-pends on whether it colocalizes with its substrate and on whether other redundant enzymes exist in the area (*34*).

Other candidate substrate neuropeptides for neprilysin in-clude somatostatin and substance P, both of which are decreased in AD brains (*5, 8*). Indeed, these peptides are good substrates for neprilysin-catalyzed proteolysis in a test-tube paradigm, and sub-stance P has been shown to be metabolically regulated by nepri-lysin in the colon under inflama-tory conditions in vivo (*35*). Incidentally, insulin-degrading enzyme (IDE), which has been most strongly implicated by some groups (*22*), has now been shown to play a relatively minor role in Aβ catabolism (see Fig. 1).

It is of interest that some substrates are known to regulate the activity/expression of neprilysin. Morphine, a mimic of opioid pep-tides, increases neprilysin enzyme activity in neutrophils in vivo (*36*), while substance P induces neprilysin mRNA expression in fibroblasts in vitro (*37*) (see **Animation 2** http://sageke.sciencemag.org/cgi/content/full/sageke;2003/3/pe3/DC1**)**. In both cases, the receptors involved are likely to be GPCRs (*4*). Such mechanisms are beneficial in main-taining neuropeptide homeostasis through the formation of negative feedback systems; ex-cessively large amounts of substrate ligands would be reduced by increased neprilysin activity, while very small amounts of ligands would have longer lifespans due to the reduced catabolic activity. We thus hypo-thesize that there may also exist a similar mechanism in the brain (see



Animation 3. Metabolic regulation of Aβ levels through modulation of a peptide ligand-receptor mechanism in the brain. We hypothesize that a peptide ligand-receptor mechanism regulates both the ligand and Aβ levels in the brain. Bases for this hypothesis include the presence of a similar mechanism in other tissues (see Animation 2).



Animation 4. Decline with aging of neprilysin expression in the dentate gyrus. We have demonstrated that neprilysin expression is prominently reduced in the outer molecular layer, the inner molecular layer, and the polymorphic cell layer of the dentate gyrus upon aging. See (*24, 39, 40*) for more details. Abbreviations: po, polymorphic cell layer; gr, granule cell layer; iml, inner molecular layer; mml, middle molecular layer; oml, outer molecular layer.

Animation 3 (http://sageke.sciencemag.org/cgi/content/full/sageke;2003/3/pe1/DC1), although we do not yet know the exact identity of the ligand-receptor system. There are several other rea-sons to predict the presence of such a mechanism in the brain besides the obser-vations outlined above. These can be sum-marized as follows: (i) neprilysin expression in the hippocampus and neocortex (a portion of the cerebral cortex that has distinct territories involved in sensory, motor, and association functions) is closely associated with interneurons (inhibitory neurons), which are the major sources of neuropeptides (*38*); (ii) interneurons possess auto- and hetero-receptors (*38*) for various ligands by which they regulate the homeostatic levels of the ligands via a modulation of the associated signal transduction mechanisms (*38*); (iii) neprilysin is present inside secretory vesicles and at presynaptic terminals (*39, 40*) and could thus modulate the intra- and inter-cellular signals generated by these peptidic ligands, which are produced in synaptic vesi-cles and released from presynapses; and (iv) the mechanism proposed above can account for the aging-dependent decline of certain neuropeptides and of neprilysin (see next paragraph).

We have reproducibly observed an aging-dependent, selective reduction of neprilysin activity/expression in the hippocampus and neocortex (*24, 39, 40*). For these experiments, neprilysin-KO mice were used as a negative control. In the hippocampus (see Fig. 3), a prominent local neprilysin reduction was de-tected in the polymorphic cell layer, the outer molecular layer, and the inner molecular layer of the dentate gyrus, a region of the hippocampal formation involved in the relay of information from the entorhinal cortex to other areas of the hippocampus primarily re-presented by the CA3 sector. Local nepri-lysin reduction was also detected in the stria-tum lucidem--a subcortical mass that has an important excitatory or inhibitory role in pro-cessing cortical signals--of the CA3 sector. The CA3 sector processes information coming in through the perforant path (see Fig. 3) and out to hippocampal CA sectors 1-3 of the ipsilateral and contralateral sides of the brain (*40*) (see **Animation 4** http://sageke.sciencemag.org/cgi/content/full/sageke;2003/3/pe1/DC1). These results indicate that aging causes a local elevation of Aβ concentrations at the presynapses in these hippocampal areas. Anatomically speaking, these areas correspond to the presynaptic terminal zones of the perforant path and mossy fibers origi-nally projecting from the entorhinal cortex (see Fig. 3), where the initial neurodegenera-tion takes place in the early stages of the onset/progression of AD (*41*). The observed decrease in neprilysin expression is not due to a loss of neurons or of presynapses, be-cause presynaptic markers remain unchanged upon aging.

It is not, however, so easy to prove or disprove the mechanism shown in **Animation 3** (http://sageke.sciencemag.org/cgi/content/full/sageke;2003/3/pe1/DC1) using in vitro paradigms, for the following reasons. Neprilysin is expressed mainly at the presynaptic membranes of interneurons in the brain (*39*). Although the number of interneurons is smaller than that of excitatory neurons in the hippocampus and neocortex, the number of presynapses formed by inter-neurons is 100 to 1000 times greater than the number of presynapses formed by excitatory neurons (*38, 42*). Unless it becomes possible to culture interneurons in a manner that would allow formation of as many pre-synapses as occurs in vivo, it will be difficult to analyze the hypothetical ligand-regulated neprilysin activity/expression mechanism in an in vitro paradigm. The most relevant as well as realistic approach would be to quanti-fy neprilysin activity and Aβ levels in the brains of genetically manipulated mice de-ficient in a candidate neuropeptide precursor, although this would take more time than the shorter-term in vitro approaches.

Hypothesis: The Mechanism of Aging-dependent Aβ Deposition Associated with Neuropeptide Metabolism in the Brain

On the basis of the presumption that the mechanism schematized in **Animation 3** (http://sageke.sciencemag.org/cgi/content/full/sageke;2003/3/pe1/DC1) also exists in the brain, as it does in some other tissues, we suggest a mechanism for the aging-depend-ent accumulation of Aβ in human brains. If we suppose that aging causes a decrease in the synthesis of a neuropeptide (*13, 43*) that happens to be a ligand involved in the regu-lation of

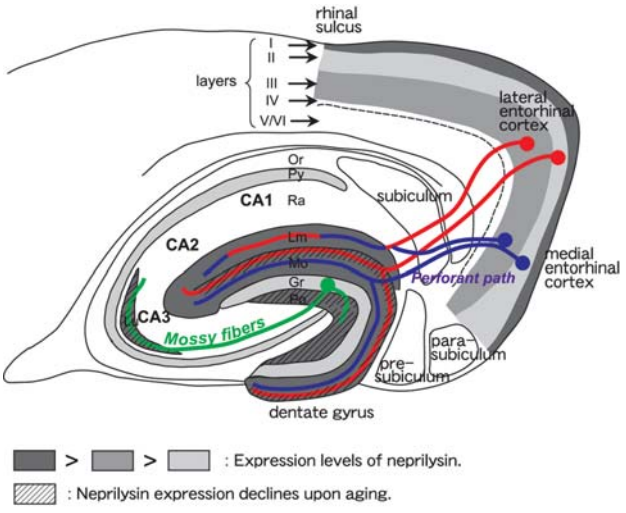
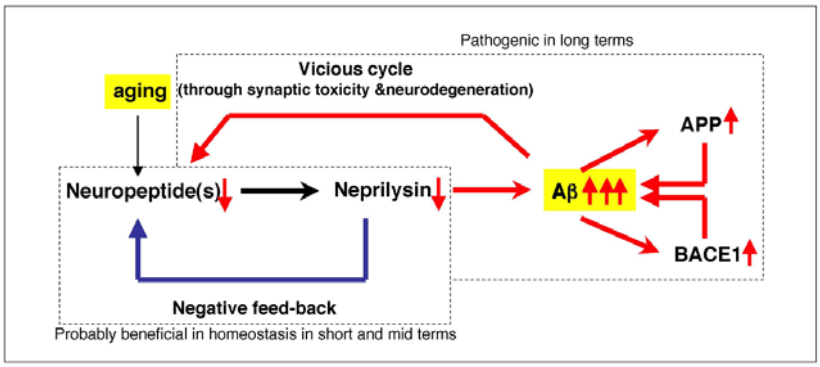
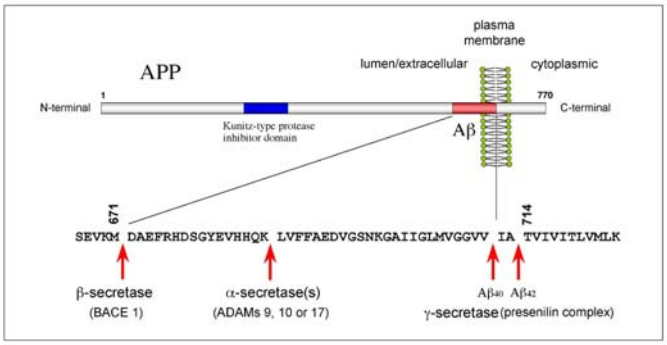


Fig. 3. Diagram of the physiological localization and aging-associated re-duction of neprilysin in the hippocampal forma-tion and entorhinal cor-tex. Previously described quantitative immunofluo-rescence observations (*24, 39, 40*) are summarized in the figure. See also Anima-tion 4. Abbreviations: Or, stratum oriens; Py, stratum pyramidale; Ra, stratum ra-diata; Lm, stratum lacuno-sum-moleculare; Mo, mole-cular layer; Gr, granule cell layer; Po,



Animation 5. Hypothesis: aging, neuropeptide, neprilysin, and Aβ metabolism. Suppose aging causes a decrease in the synthesis of a neuropeptide, which happens to be a ligand involved in the regulation of neprilysin activity/expression as well as it being a practically selective substrate for neprilysin in vivo, then the activity/expression of neprilysin will decrease in a negative feedback manner in order to maintain the homeostasis of the neuropeptide levels. Such a mechanism is probably beneficial to the brain in the relatively short terms by compensating for the reduced synthesis of the ligand peptide. An apparent adverse side effect of this negative feedback mechanism is an increase in Aβ levels particularly at presynapses. This could cause presynaptic toxicity on a long-term basis, generating a positive feed-back vicious cycle that would further lower the neuropeptide levels and elevate the Aβ levels. Further to this, the presence of two other positive feedback/vicious cycles, likely to contribute to Aβ accumulation, has been implicated. One involves an increased synthesis of APP. Another one involves an increase in the expression of β-secretase, or BACE 1.

neprilysin activity/expression, as well as a selective substrate for neprilysin in vivo, then the activity/expression of nepri-lysin will decrease in a negative feedback manner in order to maintain the homeostasis of the neuropeptide concentrations (see **Animation 5** http://sageke.sciencemag.org/cgi/content/full/sageke;2003/3/pe1/DC1). Such a mechanism would probably be bene-ficial to the brain in the short term, as it would compensate for the reduced synthesis of the ligand peptide. This would have been true particularly in the times when most human beings lived only 50 to 60 years.



Animation 6. Generation of Aβ from APP. APP first undergoes limited proteolysis catalyzed by β-secretase, or BACE 1, and then cleaved by γ-secretase, in which presenilin is an essential component, generating Aβ. A pathway alternative to β-secretase cleavage of APP is proteolysis conducted by α-secretase(s), the candidates for which include ADAMs 9, 10, and 17. The former pathway is considered to be amyloidogenic while the latter non-amyloidogenic.

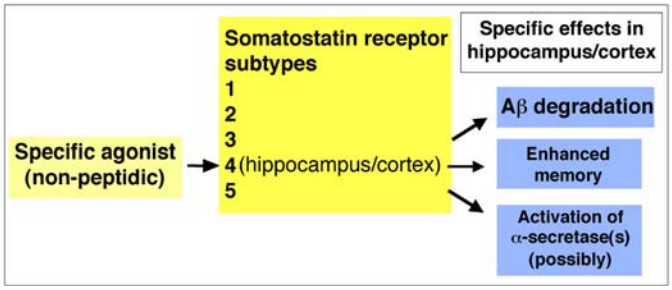
An apparent adverse side effect of this negative feedback mechanism is an increase in Aβ concentrations particularly at pre-synapses, as demonstrated by reverse gene-tics experiments using neprilysin-KO mice (*18, 39*). If Aβ □□□□□n□□□□□□□□□ remain eleva-ted long enough to cause presynaptic dys-function or degeneration, as demonstrated by a number of studies using human tissues and mouse models that overexpress APP (*44, 45*), this side effect would positively feed back on itself in a manner that would further lower the neuropeptide levels, due to a reduction in the ability of neurons to synthesize neuro-peptides. Such a process would result in a further elevation of the concentration of Aβ. The presumed presence of such a positive feedback-based, vicious cycle is mathematic-ally consistent with the fact that the accumulation of Aβ in the brain takes place very slowly in the initial presymptomatic stages of AD, but then increases gradually, and finally escalates catastrophically in what might even correspond to an essentially exponential process (*23*).

Further, two other positive feedback/ vicious cycles likely to contribute to Aβ accumulation have been described (see **Animation 5** http://sageke.sciencemag.org/cgi/content/full/sageke;2003/3/pe1/DC1). One involves an increased synthesis of APP http://sageke.sciencemag.org/cgi/genedata/sageke GdbGene;197, as observed by some investigators in AD brains (*28*). This increase is presumably associated with the neuro-protective functions of the soluble form of APP (*28*) in response to the pathological conditions present. A second process involv-es an increase in the expression of β-secretase (also known as β-site APP cleaving enzyme 1, or BACE 1) (as observed in the frontal cortex of AD patients) (*46*), indicating that the increase in BACE 1 occurs at a relatively late stage in the cascade of AD development, according to the different pathologi-cal stages of AD development established by Braak and Braak (47). See **Animation 6** http://sageke.sciencemag.org/cgi/content/full/sageke;2003/3/pe1/DC1 for the generation of Aβ by BACE 1 (β-secretase) and the presenilin complex (γ-secretase).

While none of these cyclic processes are likely to have had any apparent pathogenic effects during past epochs of the human race when most people died prior to the age of 60, taken together with an increasing life-span, they could synergistically result in the pro-gressive accumulation of Aβ in the brain and thereby contribute (in conjugation with other genetic and epigenetic risk factors) to an in-creased propensity to develop AD. This is essentially true of countries wherein the aver-age life-span has approached or is approa-ching 80 years. While provocative, it is note-worthy that no published studies on AD or AD-related topics support or contradict this hypothesis. However, it provides a logical, relevant explanation for the accumulation of Aβ with advancing age leading to the development of SAD, a disorder in which aging is by far the strongest risk factor.

A New Strategy to Control Brain Aβ Concentrations Using G Protein-coupled Receptor (GPCR) Ligand(s)

Although we have not yet identified the ligand-receptor system involved in the main-tenance of neprilysin expression/activity schematized in **Animation 3,** http://sageke.sciencemag.org/cgi/content/full/sageke;2003/3/pe1/DC1 somatostatin is a strong candidate ligand for the following reasons: (i) somato-statin precursor is highly expressed in the hippo-campus and neocortex (*43*); (ii) soma-tostatin has been shown, in a very reproduc-ible manner, to be decreased in the brains of AD patients; (iii) the somatostatin receptor subtype 4 is exclusively expressed in the hippocampus and neocortex (see next para-graph); (iv) somatostatin is an extremely good substrate for neprilysin-catalyzed pro-teolysis (*48*); (v) even in primary cultured neurons, and despite the difficulty pointed out in the fourth section of this Perspective, somatostatin seems to up-regulate neprilysin activity to some extent (*49*); (vi) somato-statin has been shown to improve long-term potentiation (LTP), a principal measure for memory, in hippocampal slices (*50*); and (vii) unlike in neutrophils, treatment of mice with morphine does not have any effect on neprilysin activity or Aβ levels in the brain (*51*), thus excluding the opioid peptides as candidate ligands.



Animation 7. Proposal of a new strategy to control brain Aβ concentrations through modulating GPCR. Although we have not yet identified the ligand-receptor system involved in the maintenance of neprilysin expression/activity, somatostatin is a strong candidate ligand for various reasons (see the main text). Because somatostatin receptor subtype 4 is exclusively expressed in the hippocampal formation and neocortex, if somatostatin is one of the major ligands in the neprilysin maintenance system, then a non-peptidic agonist specific for this receptor would selectively and specifically mobilize the ligand-receptor system only in these brain regions and thus should yield minimal systemic side effects. This strategy has three major advantages: (i) increased Aβ degradation, (ii) enhanced memory, and (iii) possibly the activation of α-secretase(s) represented by some of the ADAMs family members.

As indicated above, the type 4

somato-statin receptor subtype is exclusively ex-pressed in the hippocampus and neocortex (*52*). Therefore, if somatostatin is one of the major ligands in the neprilysin maintenance system, then a non-peptidic agonist specific for the type 4 somatostatin receptor would selectively and specifically mobilize the ligand-receptor system only in these brain regions and thus should yield minimal systemic side effects (see **Animation 7** http://sageke.sciencemag.org/cgi/content/full/sageke;2003/3/pe1/DC1). This strategy has three major advantages: (i) increased Aβ de-gradation, (ii) enhanced memory (through supplementing a factor that is beneficial to memory formation and, at the same time, lacking in AD brains), and (iii) possible activation of α-secretase(s), including the ADAM proteins (which are disintegrins and metalloproteases) (see **Animation 6** http://sageke.sciencemag.org/cgi/content/full/sageke;2003/3/pe1/DC1), as has been de-monstrated in cardiac tissues under normal condi-tions (*53*). Activation of α-secretase(s) in the brain would lead to a reduction in the synthesis of Aβ□□□□reducing the relative in-volvement of β-secretase in APP processing □□□□ (see **Animation 6** http://sageke.sciencemag.org/cgi/content/full/sageke;2003/3/pe1/DC1).

The advantages of utilizing neprilysin activity to modulate Aβ concentrations in the brain include the following: (i) neprilysin does not influence the processing of APP and other secretase substrates; (ii) neprilysin has similar K_m values for all of its substrates and thus preferentially proteolyzes a substrate in large abundance, for example, Aβ in the brains of patients with AD and with mild cognitive impairment (MCI), a precursor to AD (*16*); (iii) neprilysin degrades both extra-cellular and cell-associated Aβ (□□); (iv) neprilysin is a constitutively active enzyme and thus does not require activation of an inactive precursor; (v) unlike matrix metallo-proteinases and plasmin, neprilysin does not degrade matrix proteins and thus would not be destructive to structural proteins (*29-31*); and (vi) neprilysin degrades inflammation-associated peptides, such as substance P, and neurokinins 1 and 2 (*29-31*) and thus may attenuate neuroinflammation (see **McGeer Review** http://sageke.sciencemag.org/cgi/content/full/sageke;2002/29/re3). These properties make the use of neprilysin activity for the prevention and therapy of AD complementary to the present mainstream approaches represented by β- and γ-secretase inhibitors and Aβ vaccination (*19-21, 56*).

Future Perspectives

We predict that some of the present and future anti-Aβ approaches to treat AD, many of which are based on different strategies, will be optimally combined in a manner similar to that of the "cocktail therapy" em-ployed for the treatment of Acquired Immunodeficiency Syndrome (AIDS) (*57*). In this latter treatment protocol used world-wide, a cocktail of three different drugs suppresses disease development, whereas the use of one or two of the three agents generally fails to be effective. Moreover, combining anti-Aβ strategies with other stra-tegies, such as those that target inflammation, oxidative stress, gonadal steroid deficiencies, cholesterol mis-matabolism, tauopathies, etc., will make future prevention and therapy options for AD even more promising.

We are optimistic that the efforts of the AD research community will eventually make it possible for Aβ concentrations in human brains to be controlled. If this can be accomplished in the early stages of AD development, before massive neurodegenera-tion takes place, it will serve as a post-symptomatic form of therapy. If it becomes possible to pre-diagnose the MCI prodromal phase of AD then pre-symptomatic inter-vention can be initiated. The conversion of what has been interpreted as "normal aging" to AD via MCI appears to be a continuous process caused by the gradually accelerating accumulation of several brain pathologies, among which lesions formed by Aβ are prominent. Thus, it may even be possible to partially control certain aspects of brain aging by maintaining low Aβ concentrations throughout our lives.

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