Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

NEW INSIGHTS INTO THE ROLES OF METALLOPROTEINASES IN NEURODEGENERATION AND NEUROPROTECTION

A. J. Turner* and N. N. Nalivaeva*,†

*Institute of Molecular and Cellular Biology, Faculty of Biological Sciences, University of Leeds Leeds LS2 9JT, United Kingdom

†I. M. Sechenov Institute of Evolutionary Physiology and Biochemistry, Russian Academy of Sciences, 194223 St. Petersburg, Russia

Proteolytic enzymes constitute around 2% of the human genome and are involved in many stages of cell development from fertilization to death (apoptosis). The identification of many novel proteases from genome-sequencing programs has suggested them as potential new therapeutic targets. In addition, several well-characterized metallopeptidases were recently shown to possess new biological roles in neuroinflammation and neurodegeneration. As a result of these studies, metabolism of the neurotoxic and inflammatory amyloid peptide (Aβ) is considered as a physiologically relevant process with several metallopeptidases being suggested for the role of amyloid-degrading enzymes. These include the neprilysin (NEP) family of metalloproteinases (including its homologue endothelin-converting enzyme), insulin-degrading enzyme, angiotensin-converting enzyme, plasmin, and, possibly, some other enzymes. NEP also has a role in metabolism of sensory and inflammatory neuropeptides such as tachykinins and neurokinins. The existence of natural enzymatic mechanisms for removal of amyloid peptides has extended the therapeutic avenues in Alzheimer's disease (AD) and neurodegeneration. The proteolytic events underlying AD are highly compartmentalized in the cell and formation of amyloid peptide from its precursor molecule APP (amyloid precursor protein) takes place both within intracellular compartments and in the plasma membrane, especially in lipid raft domains. Degradation of amyloid peptide by metallopeptidases can also be both intra- and extracellular

INTERNATIONAL REVIEW OF NEUROBIOLOGY, VOL. 82
DOI: 10.1016/S0074-7742(07)82006-X

0074-7742/07 $35.00

depending on the activity of membrane-bound enzymes and their soluble partners. Soluble forms of proteases can be secreted or released from the cell surface through the activity of "sheddases"—another group of proteolytic enzymes involved in key cellular regulatory functions. The activity of proteases involved in amyloid metabolism depends on numerous factors (e.g., genetic, environmental, age), and some conditions (e.g., hypoxia and ischemia) shift the balance of amyloid metabolism toward accumulation of higher concentrations of Aβ. In this regard, regulation of the activity of amyloid-degrading enzymes should be considered as a viable strategy in neuroprotection.

I. Introduction

Proteolysis represents one of the key processes underlying biological events from fertilization through development to death. In the human genome there are more than 500 proteases and homologues (almost 2% of the genome), many of whose physiological roles are yet to be identified and which may provide potential therapeutic targets in the treatment of human disease. At present there are 70 known human hereditary diseases caused by mutations in protease-coding genes (http://www.uniovi.es/degradome/). Abnormal functioning of these genes are implicated in such pathologies as inflammatory diseases, cancer, cardiovascular diseases, and neurodegeneration. Understanding of the role of these enzymes and their evolutionary conservation is important for design of appropriate drugs and therapeutic strategies. Other eukaryotic species whose genomes have been deciphered to date also possess a very high number of protease and protease-like genes which in the case of *Drosophila melanogaster* and mouse are even higher than in human (Rawlings *et al.*, 2006). The number of species in which proteases have now been identified is approaching 3000 (http://merops.sanger.ac.uk/). Cysteine-, serine-, and metallopeptidases represent the major classes of peptidases; among these three classes, the metallopeptidase class is more consistently represented with the number of genes varying from 183 in *Drosophila* to 198 in mice (in human, 186). Two other classes have much higher diversity with the range in the number of genes for cysteine proteases from 76 in *Drosophila* to 153 in mice (Puente *et al.*, 2003). For the serine peptidases this range is even more pronounced: from 100 in *Caenorhabditis elegans* to 309 in *Drosophila*. There are a smaller number of aspartic proteinases, although some are of key therapeutic importance. This more conservative representation of metallopeptidases in the genomes of various species may reflect roles of these enzymes in more generic cellular functions and their unique catalytic properties which required water and metal ions for activation of the proteolytic (hydrolytic) process, which were carefully preserved in the course of evolution.

The metallopeptidases can be divided into 12 clans according to the type and number of metal ions required for their activity (Rawlings and Barrett, 2004). This chapter will focus on two distinct zinc metallopeptidase families belonging to one of these clans, namely MA, whose representatives require zinc for their activity and contain one or two histidine residues in the zinc binding motif. The first of these metallopeptidase families is the M13 family represented by neprilysin [or neutral endopeptidase (NEP)] and the second is the M2 family represented by angiotensin-converting enzyme [or peptidyl dipeptidase (ACE)]. Members of each of these families have served as important drug targets, particularly in cardiovascular disease and, more recently, have provided insight into mechanisms involved in neurodegeneration and neuroinflammation, especially from the point of view of processing of the amyloid precursor protein (APP) and its products in Alzheimer's disease (AD).

The discovery of ACE2 in our laboratory (Tipnis *et al.*, 2000), as a result of genomics approaches to the identification of zinc metalloproteinases, together with its critical role in cardiac and lung development and function (Crackower *et al.*, 2002; Donoghue *et al.*, 2000) and as the cell surface receptor for the severe-acute respiratory syndrome (SARS) *Coronavirus* (Li *et al.*, 2003), emphasizes the validity of this strategy for identification of novel therapeutic targets. ACE2 was also suggested to play an important role in the brain renin–angiotensin system being widely expressed in various brain areas but restricted mostly to neuronal cells (Doobay *et al.*, 2007).

II. The NEP Family

NEP, or neprilysin as it is now known, is a cell surface membrane-bound glycoprotein and zinc peptidase also known as CD10 or common acute lymphoblastic leukemia antigen (CALLA). It was originally identified as a major antigen of renal membranes over 30 years ago and, at that time, was implicated in the metabolism of insulin. However, as found later, NEP degrades only the insulin B chain *in vitro* and not the intact insulin dimer, which suggests that NEP does not have any physiological role in degradation of insulin. Moreover, another zinc metallopeptidase, insulin-degrading enzyme (IDE; insulysin), was subsequently discovered which appeared to fulfill the physiologically relevant function of insulin degradation. Whereas the highest concentrations of NEP are in the renal microvillar membrane, the first clues to its physiological roles came from studies on the metabolism of neuropeptides [especially the enkephalins and substance P (SP)] in the central nervous system where NEP is several orders of magnitude less abundant (Malfroy *et al.*, 1978; Matsas *et al.*, 1984; Relton *et al.*, 1983). It is now accepted that NEP functions to turn off neuropeptide signals

at the synapse in an analogous fashion to the hydrolysis of acetylcholine by acetylcholinesterase at cholinergic synapses (see Turner and Tanzawa, 1997 for review). Evidence *in vitro* has clearly demonstrated that synaptic membranes efficiently degrade enkephalins and SP and that NEP was the primary enzyme responsible for these events (Matsas *et al.*, 1983). Combined with the data obtained *in vivo* in rodents using potent and selective NEP inhibitors, such as phosphoramidon and thiorphan (Roques *et al.*, 1980), key roles were established for NEP in the central nervous system. Subsequently, renal NEP was shown to be the principal enzyme inactivating the vasodilator, atrial natriuretic peptide (Kenny and Stephenson, 1988), which has led to much investment in the development of NEP inhibitors, either alone or in combination with ACE inhibitors (vasopeptidase inhibitors), as drugs in the treatment of hypertension, congestive heart failure, and renal disease (Bralet and Schwartz, 2001), although concerns over their safety have been raised (Quaschning, 2005).

NEP also plays important roles in other peripheral tissues such as in chemoreception and in potentiation of the response of the carotid body to hypoxia via degradation of its preferred substrate, SP (Kumar *et al.*, 1990, 2000). NEP was also detected in the liver, lungs, muscles, fat deposits, bones, the vertebrae, articular cartilages, and synoviae (Sales *et al.*, 1991). In the bones, NEP plays a role in regulation of osteoblast and osteoclast metabolism mediated by both hormones and local bone peptide factors (Howell *et al.*, 1993; Ruchon *et al.*, 2000). In the skeletal muscles, NEP participates in regeneration of muscle fibers and there are data on its role associated with hereditary muscle disorders (Broccolini *et al.*, 2006).

The cloning of NEP also revealed its identity with the CALLA or CD10 and has implicated NEP in cancer mechanisms (Letarte *et al.*, 1988; Tran-Paterson *et al.*, 1989). For example, in human prostate cancer, NEP is dramatically downregulated (Papandreou *et al.*, 1998) allowing mitogenic peptides such as bombesin and endothelin to drive androgen-independent cell division in the prostate. The survey of the expression of NEP and the NEP homologue, endothelin-converting enzyme 1 (ECE-1), in a range of prostate cancers demonstrated that there is a certain balance between the levels of expression of NEP and ECE-1 which determines the level of malignancy of the cells and that upregulation of ECE-1 expression in metastatic cells may be indicative of its role in metastatic progression (Usmani *et al.*, 2002). Later it was shown that NEP and ECE-1 act as mediators of prostate cancer invasion via a stromal–epithelial interaction (Dawson *et al.*, 2004). This and related observations have led to suggestions that the dysregulation of the balance of NEP and ECE can lead to the disease and selective reexpression of NEP in prostate cells may provide a novel approach to the treatment of prostate cancer. This is an excellent example where two homologous peptidases counterbalance each other's actions in physiology and pathology.

NEP cleaves a wide range of substrates including SP and other tachykinin peptides, in particular neurokinin B (Fig. 1), which makes it an important player

Proinflammatory peptides cleaved by metalloproteinases

- Substance P (NEP, ACE)
- Bradykinin (NEP, ACE, ECE)
- CGRP (NEP)
- Neurokinins (NEP, ACE)
- Endothelin (NEP)
- Angiotensin II (ACE2)
- Interleukin-1β (NEP)
- Insulin (IDE)
- Insulin B chain (NEP)
- Aβ (NEP, ECE, ACE, IDE)

FIG. 1. List of proinflammatory peptides cleaved by metalloproteinases.

in the arena of inflammation (Hooper and Turner, 1985). SP is released from sensory nerves inducing neurogenic inflammation and NEP, via SP degradation, limits its effects and those of other proinflammatory peptides. There are several studies reporting the role of NEP in peripheral inflammation, for example in the skin (Scholzen and Luger, 2004; Scholzen *et al.*, 2001). Moreover, NEP but not ACE was shown to be the most important for CGRP degradation in human skin, and NEP inhibitors facilitated neurogenic inflammation in the skin (Kramer *et al.*, 2005). NEP levels were also found to be significantly decreased in the plasma and monocytes of patients with juvenile idiopathic arthritis (Simonini *et al.*, 2005). In the mouse model of intestinal inflammation caused by nematode infection NEP was demonstrated to downregulate the early onset of inflammation (Barbara *et al.*, 2003). Inhibition of NEP was found to exacerbate both experimental pancreatitis and the associated lung injury (Day *et al.*, 2005), and pretreatment with recombinant human NEP ameliorated this injury in $NEP^{-/-}$ transgenic mice (Lightner *et al.*, 2002). In this connection, upregulation of NEP is considered as a potential therapeutic approach for pancreatitis-associated lung injury, which can also be true in the case of other inflammatory diseases.

Although the role of NEP in peripheral inflammation is well characterized, there are much less data on the role of NEP in inflammatory processes in the brain. However, the data on the localization and properties of NEP in the nervous system of insects provide evidence for an evolutionarily conserved role for NEP in the inactivation of tachykinin-related peptides in the brain (Isaac and Nassel, 2003; Isaac *et al.*, 2002).

It was demonstrated that NEP might play an important role in the pathogenesis of AD due to its capacity to cleave the neurotoxic and inflammatory amyloid-β (Aβ) peptide (Iwata *et al.*, 2000) that is a primary trigger for the

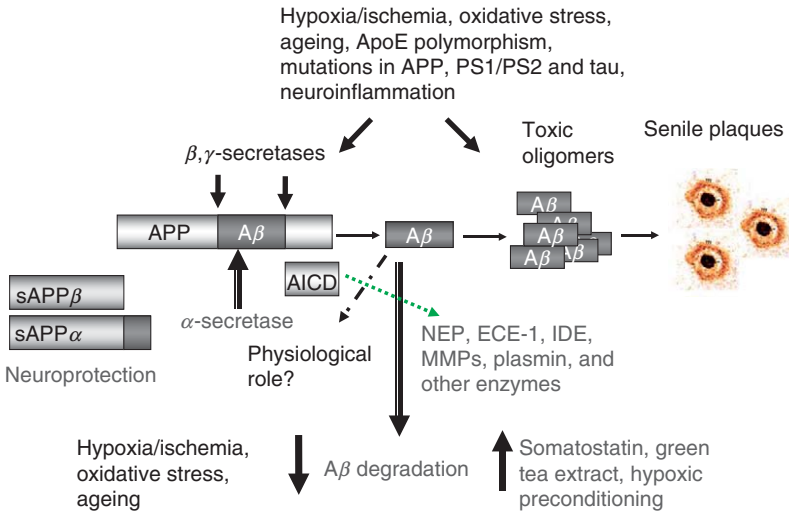

FIG. 2. Amyloidogenic processing of APP and formation of amyloid (Aβ) peptide, fibrils, and plaques. Main avenues in neurodegeneration and neuroprotection. APP, amyloid precursor protein; sAPPα, soluble product of α-secretase cleavage; sAPPβ, soluble product of β-secretase cleavage; AICD, C-terminal fragment of APP, product of γ-secretase cleavage.

development of this disease (Hardy and Higgins, 1992). It is now well documented that Aβ is formed from a large precursor molecule called APP via two consecutive cleavages (Fig. 2). The first of these cleavages occurs in the extracellular or lumenal domain and is mediated by a membrane-bound aspartic protease termed β-secretase (BACE) (Vassar and Citron, 2000). It releases a large soluble fragment of sAPPβ and the residual membrane-bound fragment. The latter is cleaved by a γ-secretase protease complex, at residues 40–42 (termed γ-site) or at residues 48–52 (termed ε-site) within the transmembrane domain. The presenilin-dependent γ-secretase and γ-site proteolytic activities are dependent on a multimeric complex of at least four different membrane proteins, including presenilin-1 (PS1) or presenilin-2 (PS2), nicastrin, Aph-1, and Pen-2 (Francis *et al.*, 2002; Yu *et al.*, 2000). In these complexes, the presenilins have been proposed as a novel type of transmembrane aspartic protease bearing the catalytic core of the γ-secretase (Wolfe *et al.*, 1999). Whereas the cleavage at the γ-site generates Aβ, the subsequent cleavage at the ε-site generates a cytosolic fragment referred to as ICD (Passer *et al.*, 2000) or AICD (*βA*PP *I*ntra*C*ellular *D*omain). The exact role of AICD remains unclear but it has been suggested to act as a functional transcriptional regulator (Cao and Sudhof, 2001) in combination with the regulatory proteins Fe65 and the chromatin-associated histone acetyltransferase, Tip60. There were reports that AICD and nicastrin regulate expression of NEP but this is controversial and still has to be

proved experimentally in animal models (Hass and Yankner, 2005; Hebert *et al.*, 2006; Pardossi-Piquard *et al.*, 2005, 2006). An additional component of the γ-secretase complex, termed TMP21, which is a member of the p24 cargo protein family, appears to differentially regulate γ-secretase cleavage without affecting ε-secretase activity (Chen *et al.*, 2006). The proteolytic events involved in processing of APP are highly compartmentalized in the cell taking place both within intracellular compartments and in the plasma membrane. The rate limiting reaction of APP cleavage by β-secretase (BACE) was shown to take place especially in lipid raft domains enriched with cholesterol and glycosphingolipids and targeting BACE to lipid rafts increased production of Aβ (Cordy *et al.*, 2003). Depletion of cell cholesterol by lovastatin resulted in a decrease in both of sAPPβ and Aβ levels in the cell culture model. This explains the positive effect of statins on the development of AD pathology observed in patients with statin treatment (Sparks *et al.*, 2006).

Under normal conditions, Aβ occurs as a soluble fragment, the concentration of which is normally tightly controlled below the threshold for its self-aggregation into β sheet fibrils (Burdick *et al.*, 1992). Until recently, production of Aβ in the brain and other tissues was thought to be an irreversible process, leading in the case of their disruption, to amyloidogenic diseases. However, in the last few years, neprilysin and several other proteases (IDE, ECE-1 and ECE-2, plasmin) have been found to be capable of degrading Aβ *in vitro* and *in vivo* (for review see Carson and Turner, 2002; Turner *et al.*, 2004). The sites of Aβ cleaved by known proteinases are shown in Fig. 3. Pathological downregulation of these enzymes and, in particular of NEP, could predispose to accumulation of Aβ and the development of AD (Apelt *et al.*, 2003; Nalivaeva *et al.*, 2004). In particular, in mice deficient in NEP or ECE-1, amyloid deposits are seen to deposit at significant levels in the brain (Eckman *et al.*, 2003; Hersh *et al.*, 2002). Furthermore, injection of amyloid-β peptide into the brains of rodents significantly enhances the concentrations of NEP mRNA and protein, suggesting the operation of a regulatory feedback mechanism to protect neurons from toxic damage (Mohajeri *et al.*, 2002). NEP levels appear to be reduced in high-plaque-bearing areas of human brain in AD and in cerebral amyloid angiopathy (Carpentier *et al.*, 2002; Yasojima *et al.*, 2001) but no association has been detected to date between polymorphisms in the NEP gene and AD (Lilius *et al.*, 2003). Clinical data suggest that ischemia and stroke predispose to development of AD (Snowdon *et al.*, 1997), and it was shown that hypoxia and ischemia lead to a decrease of NEP and ECE expression (Fisk *et al.*, 2006; Nalivaeva *et al.*, 2004). Thus, a possible therapeutic approach for treatment of AD might be a chronic upregulation of these proteinases (Fig. 4), either pharmacologically or through a gene therapy approach (Marr *et al.*, 2003; Saito *et al.*, 2005; Turner *et al.*, 2004).

Maintenance of cellular concentrations of NEP, which is rather widely distributed in the body, is critical to peptide homeostasis and its up- or down-regulation can lead to a range of pathological conditions including those of

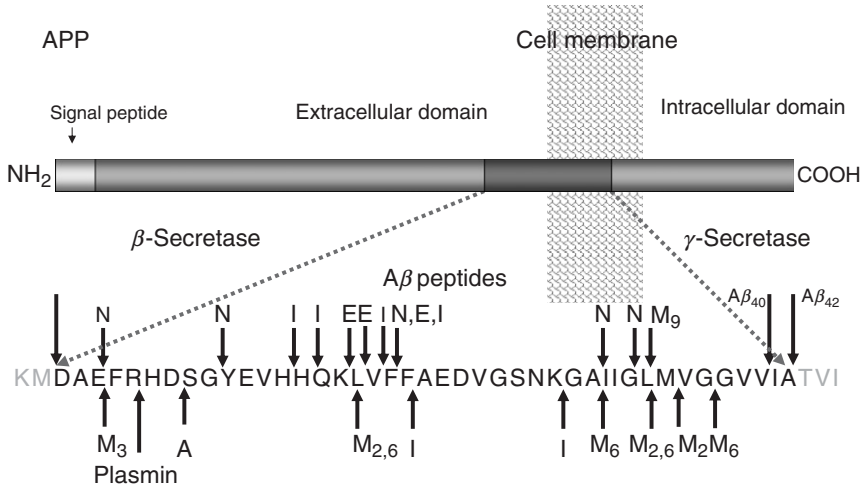

FIG. 3. Cleavage sites of Aβ-degrading enzymes. A, angiotensin-converting enzyme; E, ECE-1; I, IDE; N, NEP; M, matrix metalloproteinases (MMPs: M3, MMP-3; M2 and M6, MMP-2 and MMP-6; M9, MMP-9).

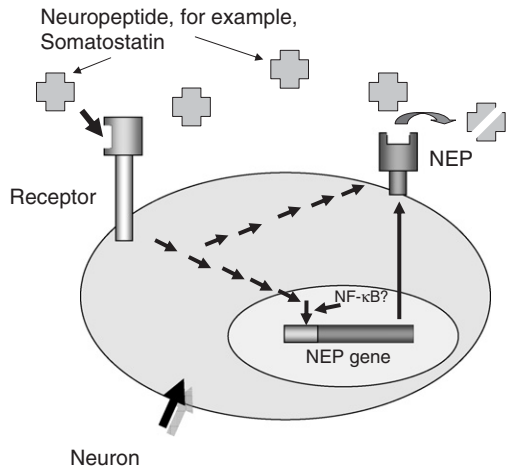

FIG. 4. Scheme of upregulation of NEP gene and its possible involvement in neuroprotection [adapted from Saito *et al.* (2003)].

cardiovascular, neurodegenerative, and tumorigenic origins. NEP was, for quite some years, a lone mammalian zinc peptidase apparently mechanistically similar to the bacterial enzyme, thermolysin, both enzymes being potently inhibited by phosphoramidon. The structural solution of the catalytic domain of human NEP

complexed with phosphoramidon (Oefner *et al.*, 2000) reveals both similarities and differences with thermolysin, and the restricted access to the active site highlights why the enzyme acts exclusively as an oligopeptidase rather than a proteinase, unlike thermolysin.

A homologue of NEP, namely neprilysin 2 (NEP2), was discovered and in the brain shown to be restricted mainly to developing and differentiated fields of the CNS. Unlike NEP and ECE-1, which are broadly expressed in the CNS and periphery, NEP2 was found to be almost exclusively expressed only in selected populations of neurons and in the spinal cord. The only peripheral areas where expression of NEP2 was detected were the pituitary and choroid plexuses. NEP2 was also found capable of degrading Aβ and its distinct localization from NEP suggests that, together with ECE-1, it may be better poised to catabolize Aβ as it is more abundantly expressed in the areas relevant to AD pathology (Facchinetti *et al.*, 2003; Thomas *et al.*, 2005).

The human genome is now known to contain at least seven NEP-related enzymes (summarized in Turner *et al.*, 2001), of which the best characterized is ECE-1, which catalyzes the final step in the biosynthesis of the potent vasoconstrictor peptide, endothelin-1 (ET-1; Matsumura *et al.*, 1990; Xu *et al.*, 1994). Several of the NEP-like enzymes are, as yet, orphan peptidases with no recognized peptide substrates. Novel strategies are urgently needed to allow the identification of physiologically relevant substrates for such newly identified proteases.

III. The NEP Homologue ECE-1

ECE-1 was first purified from rat lung (Takahashi *et al.*, 1993) but then was also found in a variety of tissues. It is most abundant in endothelial cells but is also expressed by exocrine cells, smooth muscle cells, neurons, and glia in the brain (Barnes and Turner, 1999; Barnes *et al.*, 1997; Takahashi *et al.*, 1995). To date, four isoforms of human ECE-1 differing only in a part of their N-terminal cytoplasmic region but which cleave big ETs with similar efficiencies have been characterized: named ECE-1a, ECE-1b, ECE-1c, and ECE-1d (Schweizer *et al.*, 1997; Valdenaire *et al.*, 1999). Although the relative levels of the isoform mRNA species vary between human tissues, ECE-1c mRNA is generally the predominant isoform message. There are distinct subcellular localizations for the four isoforms: whereas ECE-1a, ECE-1c, and ECE-1d proteins are localized mainly at the cell surface, ECE-1b was found to be intracellular and showed significant colocalization with a marker protein for the *trans*-Golgi network (Schweizer *et al.*, 1997). There are no significant differences in the catalytic properties between them, so it has been suggested that intracellular ECE-1 localized in Golgi and vesicles might be involved in processing of big ET whereas cell surface ECE-1 may metabolize other regulatory peptides (Turner *et al.*, 1998).

ECE-1 and its product ET-1 have been shown to be involved in such inflammatory conditions as asthma (Zhang *et al.*, 2004), chronic rhinitis with its expression in the nasal epithelium and mucosa being much higher in the case of rhinitis than in controls (Furukawa *et al.*, 1996), and idiopathic pulmonary fibrosis (Saleh *et al.*, 1997). In the latter case, an increased release of ET-1 was accompanied by increased levels of ECE-1 mRNA. Using normal bronchial epithelial cell culture, these authors have demonstrated that proinflammatory cytokines (IL-1α, IL-1β) induced a significant increase in ET-1 release and mRNA expression, while TNF-α stimulated expression of ECE-1 mRNA. In the model of acute gastric infection caused by administration of *Helicobacter pylori* lipopolysaccharide, it was also shown that the levels of ET-1 and expression of ECE-1 were significantly increased in the gastric mucosa of infected cells on day 4 but then significantly reduced (down to 60%) by day 10 following reduced gastric inflammation (Slomiany *et al.*, 2000). Developing this work further, the authors have discovered that ET-1 has also an effect on leptin production in the gastric mucosa as a consequence of ET(A) receptor activation (Slomiany and Slomiany, 2005).

Together with ET-1, ECE-1 is abundantly present in human arteries and is involved in chronic inflammation in human atherosclerosis. Upregulation of the ECE-1–ET-1 system was shown to be closely linked to the presence of chronic inflammation at the very early stages of plaque evolution and, thus, has been suggested as a target in atherosclerosis therapy (Ihling *et al.*, 2001, 2004). Plasma levels of ECE-1 were also shown to reflect the severity of ischemic complications after subarachnoid hemorrhage. The higher levels of plasma ECE-1 resulted in reduced big ET-1 and increased ET-1/big ET-1 ratio in patients who experienced symptomatic delayed cerebral ischemia, compared with other patients (Juvela, 2002). However, the levels of ECE-1 expression in rat brain cortex hemispheres and hippocampus was found to be decreased after 15-min global ischemia and restored to control levels after reperfusion (Nalivaeva *et al.*, 2004), which might reflect an adaptive reaction of the brain to increased blood supply to the affected areas.

Although ECE-1 has been regarded as a highly specific endopeptidase, it was demonstrated to be able to hydrolyze, apart from big ET-1, a number of other biologically active peptides, such as bradykinin, SP, neurotensin, angiotensin I, and insulin B chain; it is not yet clear whether any of these, or other peptides, are physiological substrates of ECE-1 (Hoang and Turner, 1997; Johnson *et al.*, 1999). It was demonstrated that ECE-1 can also degrade amyloid peptide Aβ, which made it an important player in the arena of AD (Eckman *et al.*, 2001). Overexpression of ECE-1 in Chinese hamster ovary cells, lacking endogenous ECE activity, was found to reduce extracellular Aβ concentration by up to 90% and this effect was completely abolished by treatment with a metalloproteinase inhibitor phosphoramidon. Recombinant soluble ECE-1 was shown to hydrolyze synthetic Aβ_{40} and Aβ_{42} *in vitro* at multiple sites. Comparing ECE heterozygous

and knockout mice, Eckman *et al.* (2003) showed that the concentration of Aβ peptides in the brain of these animals was elevated in comparison with control littermates in a gene-dependent manner.

IV. The ACE Family

ACE was originally identified 50 years ago as a "hypertensin-converting enzyme" (Skeggs *et al.*, 1956) and its primary substrate was identified as angiotensin I, which it converts into the vasoconstrictor angiotensin II. In parallel, it inactivates the vasodilator and inflammatory peptide bradykinin. Hence, inhibition of ACE has a powerful effect in reduction of blood pressure and the enzyme has therefore been a major cardiovascular target for many years. The catalytic activity of ACE is primarily as a "peptidyl dipeptidase," removing dipeptides from the C-terminus of a susceptible peptide substrate. In the hydrolysis of some peptides (e.g., SP, luliberin), ACE can act as an endopeptidase, although with much lower catalytic efficiency (Turner and Hooper, 2002). Mammalian ACE exists as two distinct forms, arising from the use of alternative promoters. The simplest form is germinal or testicular ACE, which is essential for male fertility and which carries a single zinc-binding and catalytic domain. Elsewhere in the body, the somatic form of ACE is duplicated and carries two active sites. The analysis of ACE X-ray structures has revealed that ACE most closely resembles a neurotensin-degrading zinc endopeptidase known as neurolysin rather than NEP, or carboxypeptidase A, on whose structure the design of ACE inhibitors was originally based (Hooper and Turner, 2003).

While seeking novel expressed sequence tags encoding zinc metallopeptidases, we identified and cloned the first human homologue of ACE (ACEH), which is now more commonly referred to as ACE2 (Tipnis *et al.*, 2000). ACE2 is also a type I integral membrane peptidase showing 40% identity and 61% similarity with ACE and conserving the critical active site residues. It contains a single catalytic domain like testicular ACE, and it is most abundantly expressed in kidney, heart, and lung (Donoghue *et al.*, 2000; Tipnis *et al.*, 2000). Physiologically, the principal role of ACE2 is thought to be the conversion of angiotensin 2 to angiotensin (1–7), which opposes the actions of ACE2. Hence ACE and ACE2 act as counterbalances in metabolism in the renin–angiotensin system. Clues to the roles of ACE2 have come from the development of mice deficient in the ACE2 gene (Crackower *et al.*, 2002). These mice have severe cardiac contractility defects, increased plasma angiotensin II levels, and an upregulation of cardiac hypoxia-related genes, implicating ACE2 as an essential regulator of heart function. Intriguingly, a double knockout in mice of both the ACE and ACE2 genes is able to rescue the cardiac defect seen with the ACE2-deficient mice (Crackower *et al.*, 2002). ACE2 mRNA and protein levels are

substantially reduced in the kidney in diabetic rats, suggesting that the enzyme may have a role in the development of diabetic complications (Tikellis *et al.*, 2004). It has been shown that ACE2 is widely distributed throughout the brain but is mainly localized to the cytoplasm of neuronal cells in the brain and not present in glia. Moreover, ACE2 levels appear to be highly regulated by the renin–angiotensin system, suggesting its involvement in this system in the brain. ACE2 expression in the brain structures involved in the control of cardiovascular function suggests that it may have a role in the central regulation of blood pressure and hypertension (Doobay *et al.*, 2007).

The most remarkable discovery in relation to ACE2 biology has been the demonstration that the enzyme functions as the receptor for the SARS virus (Li *et al.*, 2003) and numerous subsequent studies have confirmed and extended this observation. Thus, the structure of the SARS *Coronavirus* spike receptor-binding domain complexed with receptor has been determined (Li *et al.*, 2005) and compounds blocking spike protein and ACE2 interaction discovered, for example emodin (Ho *et al.*, 2007). ACE2 also appears to play a critical role in protection against acute lung injury from SARS infection, or other causes of acute respiratory distress syndrome (Imai *et al.*, 2005).

One feature that both ACE (Hooper *et al.*, 1987) and ACE2 (Lambert *et al.*, 2005) share is the ability to be shed from the plasma membrane by cleavage within the juxtamembrane region releasing the bulk of the protein, including the intact catalytic domain, into the extracellular medium. This process is common to a growing number of membrane proteins of diverse characteristics (see Hooper *et al.*, 1997 for review), an event which is generally receptor-regulated and sensitive to inhibition by a group of hydroxamate metalloproteinase inhibitors such as batimastat. This has led to the identification of members of the ADAMs (a disintegrin and metalloproteinase) family of zinc proteinases, typified by tumor necrosis factor-α converting enzyme ("TACE"; ADAM17) as candidate shedding enzymes (Allinson *et al.*, 2004). Our data suggest that NEP might be also shed from the cell surface by a similar mechanism (Fisk, L., Nalivaeva, N. N., and Turner, A. J., unpublished data). However, the detailed molecular mechanism of this phenomenon has still to be elucidated.

ACE inhibitors have been shown to reduce development of diabetes, improve surrogate markers of inflammation, and reduce cardiovascular disease and renal disease (McFarlane *et al.*, 2003). Increasing evidence indicates that systemic inflammation and neuroinflammation are central features in cerebrovascular disease and that hypertension, through the vasoactive peptides angiotensin and ET-1, promotes and accelerates the atherosclerotic process via inflammatory mechanisms (Di Napoli and Papa, 2005). It was demonstrated that in humans the product of ACE activity angiotensin II induces IL-6 production through a mineralocorticoid receptor-dependent mechanism (Luther *et al.*, 2007). This suggests that ACE inhibitors might also be considered as targets in neuroprotection.

An ACE polymorphism was demonstrated to be associated with AD in the Japanese population (Hu *et al.*, 1999), and later it was found that ACE can indeed hydrolyze Aβ *in vitro* and reduce accumulation of Aβ in cell cultures (Hu *et al.*, 2001; Oba *et al.*, 2005). However, Eckman *et al.* (2006) have demonstrated that *in vivo* ACE does not have a physiological role in clearing Aβ and it is cleaved in the brain mostly by NEP and ECE-1 since ACE-deficient mice did not demonstrate accumulation of Aβ while deficit of NEP or ECE-1 activity resulted in additive increases in brain Aβ levels.

V. Ischemia/Hypoxia and Ageing as Factors Affecting Metalloproteinases

It is becoming more obvious that neurodegeneration and development of AD can be promoted by cardiovascular lesions, ischemia and stroke (Hofman *et al.*, 1997; Kalaria, 2000). Indeed, the analysis of various autopsy series demonstrates that 60–90% of AD cases exhibit variable cerebrovascular pathology. Hypertension has also been suggested as a risk factor in development of AD (for review see Skoog and Gustafson, 2006). Taking into account that NEP and ECE-1 are involved in vascular functions and can contribute to amyloid metabolism, we have analyzed levels of expression of ECE-1 in the brain cortex of rats after 15-min global ischemia and found a significant decrease of NEP and ECE-1 protein levels in brain hemispheres and both hippocampi which returned to normal levels after 2-h reperfusion (Nalivaeva *et al.*, 2004). We have also demonstrated that NEP and ECE-1 levels were lower in the cortex and striatum of rats subjected to prenatal hypoxia (7% O_2, 3 h, 13th day of gestation) (Nalivaeva *et al.*, 2003). Preconditioning to mild (15% O_2) hypoxia before the episodes of acute hypoxia had a protective effect restoring the levels of NEP and ECE-1 in rat brain structures analyzed during the first month after birth. Using human neuroblastoma NB7 cells, we have also demonstrated that hypoxia (1.0–2.5% O_2) resulted in a decrease in expression of ECE-1 (Fisk *et al.*, 2006) and also in expression of NEP both at the protein and mRNA levels at and NEP activity (Fisk *et al.*, 2007). Moreover, both hypoxia and ischemia resulted in an increased production of sAPPβ and reduced amount of sAPPα. These data allowed us to conclude that hypoxic and ischemic conditions in the brain might lead to a shift of amyloid metabolism toward formation of higher levels of Aβ due to an increased rate of β-secretase reaction and reduced activity of such amyloid-degrading enzymes as NEP and ECE-1 (Fig. 4).

Analyzing the effect of chronic hypoxia (1%, 24 h) on expression of NEP estimated by the method of real-time PCR in rat primary cortical neurons and astrocytes, we have found that NEP mRNA levels were downregulated (by 20%) under hypoxia in neurons and upregulated in astrocytes (Fig. 5).

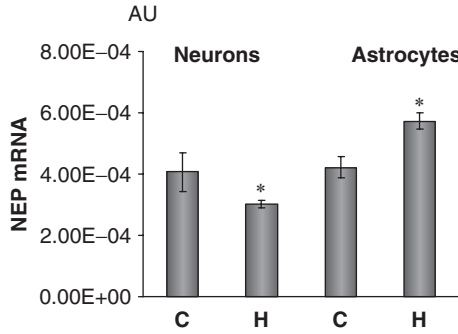

FIG. 5. Effect of hypoxia on the level of NEP mRNA expression in primary cortical neurons and astrocytes. NEP mRNA detected by real-time PCR (related to GAPDH mRNA). C, control; H, hypoxia (1% O_2, 24 h, $n = 12$). *$p < 0.05$ compared to control.

Previously, Apelt *et al.* (2003) reported NEP mRNA upregulation in amyloid plaque-surrounding reactive astrocytes in transgenic Tg2576 mice that produce human amyloid-β peptides from birth and develop amyloid-β plaques which may suggest a role of plaque-mediated astrogliosis in Aβ degradation. In our experiments, mRNA levels were also upregulated in hypoxic astrocytes, which might be an adaptive reaction of astrocytes to pathological conditions (Fisk *et al.*, 2007).

It is important to note that expression of NEP with ageing was found to decrease in the cortex and hippocampus of rats, while its levels in the striatum (where amyloid deposits have not been reported in AD brains) were as high as at the end of the first month after birth (Nalivaeva *et al.*, 2004). Decreased levels of another Aβ-degrading enzyme, IDE, have also been reported in the brain of aged mice (Caccamo *et al.*, 2005) and in rat brain structures (Nalivaeva-Turner *et al.*, 2006). Moreover, our experiments demonstrated that in the brain of rats with experimental type II diabetes IDE levels were lower than in control animals (Kochkina *et al.*, 2006). These data suggest that age-related deficit of amyloid-degrading enzymes might be one of the factors leading to the development of the sporadic form of AD and upregulation of NEP and other Aβ-degrading enzymes become one of the possible therapeutic targets in neurodegeneration and AD.

Several physiological and pharmaceutical ways of upregulation of the neprilysin gene have been suggested. One of the approaches, suggested by Saito *et al.* (2003), involves NEP substrates as molecules activating expression of the NEP gene via a positive feedback mechanism and as yet unknown signaling pathways (Fig. 4). Analyzing various NEP substrates in this experimental paradigm, these authors (Saito *et al.*, 2005) were able to demonstrate that only somatostatin was capable to stimulate NEP activity in primary cortical neurons. Since somatostatin levels in the brain decrease with age these results indicate that age-related

downregulation of somatostatin expression could be one of the triggers for Aβ accumulation leading to late-onset sporadic AD. In our experiments using human neuroblastoma cells expressing NEP and ECE-1, we were not able to detect somatostatin-dependent upregulation of NEP mRNA or activity while ECE-1 was significantly upregulated by this peptide in a dose-dependent manner (Fisk, L., Nalivaeva, N. N., and Turner, A. J., unpublished data).

Another physiological pathway of regulation of NEP gene has been suggested by Checler and colleagues who demonstrated that the C-terminal product of APP cleavage by γ-secretase (AICD) was able to transactivate the NEP gene promoter activating NEP expression and that presenilin molecules (PS1 and PS2) and the protein nicastrin in the γ-secretase complex were essential for this effect (Pardossi-Piquard *et al.*, 2005, 2006).

Among chemical compounds that can upregulate NEP expression, green tea extract (EFLA85942) has been demonstrated as effective in human neuroblastoma SK-N SH cells (Ayoub and Melzig, 2006; Melzig and Janka, 2003). According to our observations, the active compound of the green tea extract, namely, polyphenol (−)-epigallocatechin-3-gallate stimulates in a dose-dependent manner both NEP expression at protein level and its activity in human neuroblastoma NB7 cells (Fisk, L., Nalivaeva, N. N., and Turner, A. J., unpublished data).

VI. Conclusions

In this chapter, several examples have demonstrated the role of metalloproteinases in neurodegeneration, neuroprotection, and neuroinflammation. This was mostly shown for NEP, ECE-1, and ACE; however, there are other enzymes whose targeting might be beneficial for prevention of neurological disorders. Among them are the enzymes of amyloidogenic processing of APP by β-secretase (and subsequently by γ-secretase) and nonamyloidogenic APP processing by α-secretase (for review see Cordy *et al.*, 2006; Neve, 2003). Although both α- and β-secretases produce neurotrophic fragments of APP (sAPPα and sAPPβ) and are believed to play a role in normal functioning of neuronal cells (Thornton *et al.*, 2006; Turner *et al.*, 2003), the α-secretase pathway prevents production of Aβ and thus is regarded as neuroprotective. Downregulation of α-secretase or upregulation of β-secretase activity will lead to changes in the balance of production of Aβ toward its accumulation and there are emerging experimental data that this might be the case in ischemic or hypoxic brain (Nalivaeva *et al.*, 2004; Wen *et al.*, 2004). There are also some data that the same compounds can activate both α-secretase and NEP activity, for example green tea extracts (Levites *et al.*, 2003; Melzig and Janka, 2003), suggesting that neuroprotective therapies might target several points in pathogenic processes. However, there also might be

situations when one compound upregulates one neuroprotective pathway and downregulates another. For example, the phorbol ester PMA was shown to activate the α-secretase pathway (Zhu *et al.*, 2001) but inhibit expression of ECE-1 (Fisk *et al.*, 2006). This observation implies the necessity of differential analysis of the effects of potentially effective neuroprotective drugs on various enzymes participating in amyloid metabolism.

Metalloproteinases represent important therapeutic targets not only in neurodegeneration but also in cardiovascular diseases and prostate cancer, and understanding their intricate interrelationship is still one of the most fascinating areas of modern molecular and cell biology.

References

Allinson, T., Parkin, E., Turner, A. J., and Hooper, N. M. (2004). The role of ADAM10 and ADAM17 in the ectodomain shedding of angiotensin converting enzyme and the amyloid precursor protein. *Eur. J. Biochem.* **271,** 2539–2547.

Apelt, J., Ach, K., and Schliebs, R. (2003). Aging-related down-regulation of neprilysin, a putative β-amyloid-degrading enzyme, in transgenic Tg2576 Alzheimer-like mouse brain is accompanied by an astroglial upregulation in the vicinity of β-amyloid plaques. *Neurosci. Lett.* **339,** 183–186.

Ayoub, S., and Melzig, M. F. (2006). Induction of neutral endopeptidase (NEP) activity of SK-N-SH cells by natural compounds from green tea. *J. Pharm. Pharmacol.* **58,** 495–501.

Barbara, G., De Giorgio, R., Stanghellini, V., Corinaldesi, R., Cremon, C., Gerard, N., Gerard, C., Grady, E. F., Bunnett, N. W., Blennerhassett, P. A., and Collins, S. M. (2003). Neutral endopeptidase (EC 3. 4. 24. 11) downregulates the onset of intestinal inflammation in the nematode infected mouse. *Gut* **52,** 1457–1464.

Barnes, K., and Turner, A. J. (1999). Endothelin converting enzyme is located on β-actin filaments in smooth muscle cells. *Cardiovasc. Res.* **42,** 814–822.

Barnes, K., Walkden, B. J., Wilkinson, T. C., and Turner, A. J. (1997). Expression of endothelin-converting enzyme in both neuroblastoma and glial cell lines and its localization in rat hippocampus. *J. Neurochem.* **68,** 570–577.

Bralet, J., and Schwartz, J.-C. (2001). Vasopeptidase inhibitors: An emerging class of cardiovascular drugs. *Trends Pharmacol. Sci.* **22,** 106–109.

Broccolini, A., Gidaro, T., Morosetti, R., Gliubizzi, C., Servidei, T., Pescatori, M., Tonali, P. A., Ricci, E., and Mirabella, M. (2006). Neprilysin participates in skeletal muscle regeneration and is accumulated in abnormal muscle fibres of inclusion body myositis. *J. Neurochem.* **96,** 777–789.

Burdick, D., Soreghan, B., Kwon, M., Kosmoski, J., Knauer, M., Henschen, A., Yates, J., Cotman, C., and Glabe, C. (1992). Assembly and aggregation properties of synthetic Alzheimer's A4/amyloid peptide analogs. *J. Biol. Chem.* **267,** 546–554.

Caccamo, A., Oddo, S., Sugarman, M. C., Akbari, Y., and LaFerla, F. M. (2005). Age- and region-dependent alterations in Aβ-degrading enzymes: Implications for Aβ-induced disorders. *Neurobiol. Aging* **26,** 645–654.

Cao, X., and Sudhof, T. C. (2001). A transcriptionally active complex of APP with Fe65 and histone acetyltransferase Tip60. *Science* **293,** 115–120.

Carpentier, M., Robitaille, Y., DesGroseillers, L., Boileau, G., and Marcinkiewicz, M. (2002). Declining expression of neprilysin in Alzheimer disease vasculature: Possible involvement in cerebral amyloid angiopathy. *J. Neuropathol. Exp. Neurol.* **61,** 849–856.

Carson, J. A., and Turner, A. J. (2002). β-amyloid catabolism: Roles for neprilysin (NEP) and other metallopeptidases? *J. Neurochem.* **81,** 1–8.

Chen, F., Hasegawa, H., Schmitt-Ulms, G., Kawarai, T., Bohm, C., Katayama, T., Gu, Y., Sanjo, N., Glista, M., Rogaeva, E., Wakutani, Y., Pardossi-Piquard, R., *et al.* (2006). TMP21 is a presenilin complex component that modulates γ-secretase but not ε-secretase activity. *Nature* **440,** 1208–1212.

Cordy, J. M., Hussain, I., Dingwall, C., Hooper, N. M., and Turner, A. J. (2003). Exclusively targeting β-secretase to lipid rafts by GPI-anchor addition up-regulates beta-site processing of the amyloid precursor protein. *Proc. Natl. Acad. Sci. USA* **100,** 11735–11740.

Cordy, J. M., Hooper, N. M., and Turner, A. J. (2006). The involvement of lipid rafts in Alzheimer's disease. *Mol. Membr. Biol.* **23,** 111–122.

Crackower, M. A., Sarao, R., Oudit, G. Y., Yagil, C., Kozieradzki, I., Scanga, S. E., Oliveira-dos-Santos, A. J., da Costa, J., Zhang, L., Pei, Y., Scholey, J., Ferrario, C. M., *et al.* (2002). Angiotensin-converting enzyme 2 is an essential regulator of heart function. *Nature* **417,** 799–802.

Dawson, L. A., Maitland, N. J., Turner, A. J., and Usmani, B. A. (2004). Stromal-epithelial interactions influence prostate cancer cell invasion by altering the balance of metallopeptidase expression. *Br. J. Cancer* **90,** 1577–1582.

Day, A. L., Wick, E., Jordan, T. H., Jaffray, C. E., Bunnett, N. W., Grady, E. F., and Kirkwood, K. S. (2005). Neutral endopeptidase determines the severity of pancreatitis-associated lung injury. *J. Surg. Res.* **128,** 21–27.

Di Napoli, M., and Papa, F. (2005). Inflammation, blood pressure, and stroke: An opportunity to target primary prevention? *Curr. Hypertens. Rep.* **7,** 44–51.

Doobay, M. F., Talman, L. S., Obr, T. D., Tian, X., Davisson, R. L., and Lazartigues, E. (2007). Differential expression of neuronal ACE2 in transgenic mice with overexpression of the brain renin-angiotensin system. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **292,** R373–381.

Donoghue, M., Hsieh, F., Baronas, E., Godbout, K., Gosselin, M., Stagliano, N., Donovan, M., Woolf, B., Robison, K., Jeyaseelan, R., Breitbart, R. E., and Acton, S. (2000). A novel angiotensin-converting enzyme-related carboxypeptidase (ACE2) converts angiotensin I to angiotensin 1–9. *Circ. Res.* **87,** 1–9.

Eckman, E. A., Reed, D. K., and Eckman, C. B. (2001). Degradation of the Alzheimer's amyloid β peptide by endothelin-converting enzyme. *J. Biol. Chem.* **276,** 24540–24548.

Eckman, E. A., Watson, M., Marlow, L., Sambamurti, K., and Eckman, C. B. (2003). Alzheimer's disease β-amyloid peptide is increased in mice deficient in endothelin-converting enzyme. *J. Biol. Chem.* **278,** 2081–2084.

Eckman, E. A., Adams, S. K., Troendle, F. J., Stodola, B. A., Kahn, M. A., Fauq, A. H., Xiao, H. D., Bernstein, K. E., and Eckman, C. B. (2006). Regulation of steady-state β-amyloid levels in the brain by neprilysin and endothelin-converting enzyme but not angiotensin-converting enzyme. *J. Biol. Chem.* **281,** 30471–30478.

Facchinetti, P., Rose, C., Schwartz, J. C., and Ouimet, T. (2003). Ontogeny, regional and cellular distribution of the novel metalloprotease neprilysin 2 in the rat: A comparison with neprilysin and endothelinconverting enzyme-1. *NeuroScience* **118,** 627–639.

Fisk, L., Nalivaeva, N. N., and Turner, A. J. (2006). Regulation of endothelin-converting enzyme-1 expression in human neuroblastoma cells. *Exp. Biol. Med.* **231,** 1048–1053.

Fisk, L., Nalivaeva, N. N., Boyle, J. P., Peers, C. S., and Turner, A. J. (2007). Effects of hypoxia and oxidative stress on expression of neprilysin in human neuroblastoma cells and rat cortical neurons and astro cytes. *Neurochem. Res.* May 7, 2007 [Epub ahead of print].

Francis, R., McGrath, G., Zhang, J., Ruddy, D. A., Sym, M., Apfeld, J., Nicoll, M., Maxwell, M., Hai, B., Ellis, M. C., Parks, A. L., Xu, W., *et al.* (2002). Aph-1 and pen-2 are required for Notch pathway signaling, γ-secretase cleavage of APP, and presenilin protein accumulation. *Dev. Cell* **3,** 85–97.

Furukawa, K., Saleh, D., Bayan, F., Emoto, N., Kaw, S., Yanagisawa, M., and Giaid, A. (1996). Co-expression of endothelin-1 and endothelin-converting enzyme-1 in patients with chronic rhinitis. *Am. J. Respir. Cell Mol. Biol.* **14,** 248–253.

Hardy, J. A., and Higgins, G. A. (1992). Alzheimer's disease: The amyloid cascade hypothesis. *Science* **256,** 184–185.

Hass, M. R., and Yankner, B. A. (2005). A γ-secretase-independent mechanism of signal transduction by the amyloid precursor protein. *J. Biol. Chem.* **280,** 36895–36904.

Hebert, S. S., Serneels, L., Tolia, A., Craessaerts, K., Derks, C., Filippov, M. A., Muller, U., and De Strooper, B. (2006). Regulated intramembrane proteolysis of amyloid precursor protein and regulation of expression of putative target genes. *EMBO Rep.* **7,** 739–745.

Hersh, L. B., Yu, J., Zhu, H., Gao, J. T., Mucke, L., and Kindy, M. S. (2002). Neprilysin regulation of amyloid deposition in Alzheimer's disease. *J. Neurochem.* **81**(Suppl. 1), 67.

Ho, T. Y., Wu, S. L., Chen, J. C., Li, C. C., and Hsiang, C. Y. (2007). Emodin blocks the SARS coronavirus spike protein and angiotensin-converting enzyme 2 interaction. *Antiviral Res.* **74,** 92–101.

Hoang, M. V., and Turner, A. J. (1997). Novel activity for endothelin-converting enzyme: Hydrolysis of bradykinin. *Biochem. J.* **327,** 23–26.

Hofman, A., Ott, A., Breteler, M. M., Bots, M. L., Slooter, A. J., Van Harskamp, F., van Duijn, C. N., Van Broeckhoven, C., and Grobbee, D. E. (1997). Atherosclerosis, apolipoprotein E, and prevalence of dementia and Alzheimer's disease in the Rotterdam Study. *Lancet* **349,** 151–154.

Hooper, N. M., and Turner, A. J. (1985). Neurokinin B is hydrolysed by synaptic membranes and by endopeptidase-24.11 (enkephalinase) but not by angiotensin converting enzyme. *FEBS Lett.* **190,** 133–136.

Hooper, N. M., and Turner, A. J. (2003). An ACE structure. *Nat. Struct. Biol.* **10,** 155–157.

Hooper, N. M., Keen, J., Pappin, D. J. C., and Turner, A. J. (1987). Pig kidney angiotensin converting enzyme. Purification and characterization of amphipathic and hydrophilic forms of the enzyme establishes C-terminal anchorage to the plasma membrane. *Biochem. J.* **247,** 85–93.

Hooper, N. M., Karran, E. H., and Turner, A. J. (1997). Membrane protein secretases. *Biochem. J.* **321,** 265–279.

Howell, S., Caswell, A. M., Kenny, A. J., and Turner, A. J. (1993). Membrane peptidases on human osteoblast-like cells in culture: Hydrolysis of calcitonin and hormonal regulation of endopeptidase-24.11. *Biochem. J.* **290,** 159–164.

Hu, J., Miyatake, F., Aizu, Y., Nakagawa, H., Nakamura, S., Tamaoka, A., Takahashi, R., Urakami, K., and Shoji, M. (1999). Angiotensin-converting enzyme genotype is associated with Alzheimer disease in the Japanese population. *Neurosci. Lett.* **277,** 65–67.

Hu, J., Igarashi, A., Kamata, M., and Nakagawa, H. (2001). Angiotensin-converting enzyme degrades Alzheimer amyloid β-peptide (Aβ); retards Aβ aggregation, deposition, fibril formation; and inhibits cytotoxicity. *J. Biol. Chem.* **276,** 47863–47868.

Ihling, C., Szombathy, T., Bohrmann, B., Brockhaus, M., Schaefer, H. E., and Loeffler, B. M. (2001). Coexpression of endothelin-converting enzyme-1 and endothelin-1 in different stages of human atherosclerosis. *Circulation* **104,** 864–869.

Ihling, C., Bohrmann, B., Schaefer, H. E., Technau-Ihling, K., and Loeffler, B. M. (2004). Endothelin-1 and endothelin converting enzyme-1 in human atherosclerosis–novel targets for pharmacotherapy in atherosclerosis. *Curr. Vasc. Pharmacol.* **2,** 249–258.

Imai, Y., Kuba, K., Rao, S., Huan, Y., Guo, F., Guan, B., Yang, P., Sarao, R., Wada, T., Leong-Poi, H., Crackower, M. A., Fukamizu, A., *et al.* (2005). Angiotensin-converting enzyme 2 protects from severe acute lung failure. *Nature* **436,** 112–116.

Isaac, R. E., and Nassel, D. R. (2003). Identification and localization of a neprilysin-like activity that degrades tachykinin-related peptides in the brain of the cockroach, *Leucophaea maderae*, and locust, *Locusta migratoria*. *J. Comp. Neurol.* **457,** 57–66.

Isaac, R. E., Parkin, E. T., Keen, J. N., Nassel, D. R., Siviter, R. J., and Shirras, A. D. (2002). Inactivation of a tachykinin-related peptide: Identification of four neuropeptide-degrading enzymes in neuronal membranes of insects from four different orders. *Peptides* **23,** 725–733.

Iwata, N., Tsubuki, S., Takaki, Y., Watanabe, K., Seikiguchi, M., Hosoki, E., Kawashima-Morishima, M., Lee, H.-J., Hama, E., Sekine-Aizawa, Y., and Saido, T. C. (2000). Identification of the major Aβ1–42-degrading catabolic pathway in brain parenchyma: Suppression leads to biochemical and pathological deposition. *Nat. Med.* **6,** 143–150.

Johnson, G. D., Stevenson, T., and Ahn, K. (1999). Hydrolysis of peptide hormones by endothelin-converting enzyme-1. A comparison with neprilysin. *J. Biol. Chem.* **274,** 4053–4058.

Juvela, S. (2002). Plasma endothelin and big endothelin concentrations and serum endothelin-converting enzyme activity following aneurysmal subarachnoid hemorrhage. *J. Neurosurg.* **97,** 1287–1293.

Kalaria, R. N. (2000). The role of cerebral ischemia in Alzheimer's disease. *Neurobiol. Aging* **21,** 321–330.

Kenny, A. J., and Stephenson, S. L. (1988). Role of endopeptidase-24.11 in the inactivation of atrial natriuretic peptide. *FEBS Lett.* **232,** 1–8.

Kochkina, E. G., Plesneva, S. A., Zhuravin, I. A., Turner, A. J., and Nalivaeva, N. N. (2006). Study of insulin-degrading enzyme expression in the brain and peripheral organs of hypoxic and diabetic rats 11th meeting of Czech and Slovak Neurochemical Society, Martin, Slovakia, 54.

Kramer, H. H., Schmidt, K., Leis, S., Schmelz, M., Sommer, C., and Birklein, F. (2005). Inhibition of neutral endopeptidase (NEP) facilitates neurogenic inflammation. *Exp. Neurol.* **195,** 179–184.

Kumar, G. K., Runold, M., Ghai, R. D., Cherniack, N. S., and Prabhakar, N. R. (1990). Occurrence of neutral endopeptidase activity in the cat carotid body and its significance in chemoreception. *Brain Res.* **517,** 341–343.

Kumar, G. K., Yu, R. K., Overholt, J. L., and Prabhakar, N. R. (2000). Role of substance P in neutral endopeptidase modulation of hypoxic response of the carotid body. *Adv. Exp. Med. Biol.* **475,** 705–713.

Lambert, D. W., Yarski, M., Warner, F. J., Thornhill, P., Parkin, E. T., Smith, A. I., Hooper, N. M., and Turner, A. J. (2005). Tumor necrosis factor-alpha convertase (ADAM17) mediates regulated ectodomain shedding of the severe-acute respiratory syndrome-coronavirus (SARS-CoV) receptor, angiotensin-converting enzyme-2 (ACE2). *J. Biol. Chem.* **280,** 30113–30119.

Letarte, M., Vera, S., Tran, R., Addis, J. B., Onizuka, R. J., Quackenbush, E. J., Jongeneel, C. V., and McInnes, R. R. (1988). Common acute lymphocytic leukemia antigen is identical to neutral endopeptidase. *J. Exp. Med.* **168,** 1247–1253.

Levites, Y., Amit, T., Mandel, S., and Youdim, M. B. (2003). Neuroprotection and neurorescue against Aβ toxicity and PKC-dependent release of nonamyloidogenic soluble precursor protein by green tea polyphenol (–)-epigallocatechin-3-gallate. *FASEB J.* **17,** 952–954.

Li, F., Li, W., Farzan, M., and Harrison, S. C. (2005). Structure of SARS coronavirus spike receptor-binding domain complexed with receptor. *Science* **309,** 1864–1868.

Li, W., Moore, M. J., Vasilieva, N., Sui, J., Wong, S. K., Berne, M. A., Somasundaran, M., Sullivan, J. L., Luzuriaga, K., Greenough, T. C., Choe, H., and Farzan, M. (2003). Angiotensin-converting enzyme 2 is a functional receptor for the SARS coronavirus. *Nature* **426,** 450–454.

Lightner, A. M., Jordan, T. H., Bunnett, N. W., Grady, E. F., and Kirkwood, K. S. (2002). Recombinant human neutral endopeptidase ameliorates pancreatic elastase-induced lung injury. *Surgery* **132,** 193–199.

Lilius, L., Forsell, C., Axelman, K., Winblad, B., Graff, C., and Tjernberg, L. (2003). No association between polymorphisms in the neprilysin promoter region and Swedish Alzheimer's disease patients. *Neurosci. Lett.* **337,** 111–113.

Luther, J. M., Gainer, J. V., Murphey, L. J., Yu, C., Vaughan, D. E., Morrow, J. D., and Brown, N. J. (2007). Angiotensin II induces interleukin-6 in humans through a mineralocorticoid receptor-dependent mechanism. *Hypertension* **48,** 1050–1057.

Malfroy, B., Swerts, J. P., Guyon, A., Roques, B. P., and Schwartz, J.-C. (1978). High-affinity enkephalin-degrading peptidase in brain is increased after morphine. *Nature* **276,** 523–526.

Marr, R. A., Rockenstein, E., Mukherjee, A., Kindy, M. S., Hersh, L. B., Gage, F. H., Verma, I. M., and Masliah, E. (2003). Neprilysin gene transfer reduces human amyloid pathology in transgenic mice. *J. Neurosci.* **23,** 1992–1996.

Matsas, R., Fulcher, I. S., Kenny, A. J., and Turner, A. J. (1983). Substance P and [Leu]enkephalin are hydrolyzed by an enzyme in pig caudate synaptic membranes that is identical with the endopeptidase of kidney microvilli. *Proc. Natl. Acad. Sci. USA* **80,** 3111–3115.

Matsas, R., Turner, A. J., and Kenny, A. J. (1984). The metabolism of neuropeptides. The hydrolysis of peptides, including enkephalins, tachykinins and their analogues, by endopeptidase-24.11. *Biochem. J.* **223,** 433–440.

Matsumura, Y., Ikegawa, R., Takaoka, M., and Morimoto, S. (1990). Conversion of porcine big endothelin to endothelin by an extract from the porcine aortic endothelial cells. *Biochem. Biophys. Res. Commun.* **167,** 203–210.

McFarlane, S. I., Kumar, A., and Sowers, J. R. (2003). Mechanisms by which angiotensin-converting enzyme inhibitors prevent diabetes and cardiovascular disease. *Am. J. Cardiol.* **91,** 30H–37H.

Melzig, M. F., and Janka, M. (2003). Enhancement of neutral endopeptidase activity in SK-N-SH cells by green tea extract. *Phytomedicine* **10,** 494–498.

Mohajeri, M. H., Wollmer, M. A., and Nitsch, R. M. (2002). Aβ42-induced increase in neprilysin is associated with prevention of amyloid plaque formation *in vivo*. *J. Biol. Chem.* **277,** 35460–35465.

Nalivaeva, N. N., Fisk, L., Canet Aviles, R. M., Plesneva, S. A., Zhuravin, I. A., and Turner, A. J. (2003). Effects of prenatal hypoxia on expression of amyloid precursor protein and metallopeptidases in the rat brain. *Lett. Peptide Sci.* **10,** 455–462.

Nalivaeva, N. N., Fisk, L., Kochkina, E. G., Plesneva, S. A., Zhuravin, I. A., Babusikova, E., Dobrota, D., and Turner, A. J. (2004). Effect of hypoxia/ischemia and hypoxic preconditioning/reperfusion on expression of some amyloid-degrading enzymes. *Ann. NY Acad. Sci.* **1035,** 21–33.

Nalivaeva-Turner, N. N., Fisk, L., Kochkina, E. G., Makova, N., Plesneva, S. A., Zhuravin, I. A., and Turner, A. J. (2006). Developmental dynamics of amyloid-degrading enzymes in the rat brain under normal and hypoxic conditions. Proceedings of the 5th FENS Forum, Vienna, Austria, July 8–12, 2006. *FENS Abstr.* **3**, A130.30.

Neve, R. L. (2003). A new wrestler in the battle between α- and β-secretases for cleavage of APP. *Trends Neurosci.* **26,** 461–463.

Oba, R., Igarashi, A., Kamata, M., Nagata, K., Takano, S., and Nakagawa, H. (2005). The N-terminal active centre of human angiotensin-converting enzyme degrades Alzheimer amyloid β-peptide. *Eur. J. Neurosci.* **21,** 733–740.

Oefner, C., D'Arcy, A., Nennig, M., Winkler, F. K., and Dale, G. E. (2000). Structure of human neutral endopeptidase (Neprilysin) complexed with phosphoramidon. *J. Mol. Biol.* **296,** 341–349.

Papandreou, C. N., Usmani, B., Geng, Y., Bogenrieder, T., Freeman, R., Wilk, S., Finstad, C. L., Reuter, V. E., Powell, C. T., Scheinberg, D., Magill, C., Scher, H. I., *et al.* (1998). Neutral endopeptidase 24.11 loss in metastatic human prostate cancer contributes to androgen-independent progression. *Nat. Med.* **4,** 50–57.

Pardossi-Piquard, R., Petit, A., Kawarai, T., Sunyach, C., Alves da Costa, C., Vincent, B., Ring, S., D'Adamio, L., Shen, J., Muller, U., St. George-Hyslop, P., and Checler, F. (2005). Presenilin-dependent transcriptional control of the Aβ-degrading enzyme neprilysin by intracellular domains of APP and APLP. *Neuron* **46,** 541–554.

Pardossi-Piquard, R., Dunys, J., Yu, G., St. George-Hyslop, P., Alves da Costa, C., and Checler, F. (2006). Neprilysin activity and expression are controlled by nicastrin. *J. Neurochem.* **97,** 1052–1056.

Passer, B., Pellegrini, L., Russo, C., Siegel, R. M., Lenardo, M. J., Schettini, G., Bachmann, M., Tabaton, M., and D'Adamio, L. (2000). Generation of an apoptotic intracellular peptide by gamma-secretase cleavage of Alzheimer's amyloid protein precursor. *J. Alzheimers Dis.* **2,** 289–301.

Puente, X. S., Sanchez, L. M., Overall, C. M., and Lopez-Otin, C. (2003). Human and mouse proteases: A comparative genomic approach. *Nat. Rev. Genet.* **4,** 544–558.

Quaschning, T. (2005). Vasopeptidase inhibition for blood pressure control: Emerging experience. *Curr. Pharm. Des.* **11,** 3293–3299.

Rawlings, N. D., and Barrett, A. J. (2004). Intruduction: Metallopeptidases and their clans. *In* "Handbook of Proteolytic Enzymes" (A. J. Barrett, N. D. Rawlings, and J. F. Woessner, Eds.), pp. 231–268. Elsevier Academic Press, London.

Rawlings, N. D., Morton, F. R., and Barrett, A. J. (2006). *MEROPS*: The peptidase database. *Nucleic Acids Res.* **34,** D270–D272.

Relton, J. M., Gee, N. S., Matsas, R., Turner, A. J., and Kenny, A. J. (1983). Purification of endopeptidase-24.11 ('enkephalinase') from pig brain by immunoadsorbent chromatography. *Biochem. J.* **215,** 519–523.

Roques, B. P., Fournié-Zaluski, M.-C., Soroca, E., Lecomte, J.-M., Malfroy, B., Llorens-Cortes, C., and Schwartz, J.-C. (1980). The enkephalinase inhibitor thiorphan shows antinociceptive activity in mice. *Nature* **288,** 286–288.

Ruchon, A. F., Marcinkiewicz, M., Ellefsen, K., Basak, A., Aubin, J., Crine, P., and Boileau, G. (2000). Cellular localization of neprilysin in mouse bone tissue and putative role in hydrolysis of osteogenic peptides. *J. Bone Miner. Res.* **15,** 1266–1274.

Saito, T., Takaki, Y., Iwata, N., Trojanowski, J., and Saido, T. C. (2003). Alzheimer's disease, neuropeptides, neuropeptidase, and amyloid-β peptide metabolism. *Sci. Aging Knowledge Environ.* **3,** E1.

Saito, T., Iwata, N., Tsubuki, S., Takaki, Y., Takano, J., Huang, S. M., Suemoto, T., Higuchi, M., and Saido, T. C. (2005). Somatostatin regulates brain amyloid-β peptide A42 through modulation of proteolytic degradation. *Nat. Med.* **11,** 434–439.

Saleh, D., Furukawa, K., Tsao, M. S., Maghazachi, A., Corrin, B., Yanagisawa, M., Barnes, P. J., and Giaid, A. (1997). Elevated expression of endothelin-1 and endothelin-converting enzyme-1 in idiopathic pulmonary fibrosis: Possible involvement of proinflammatory cytokines. *Am. J. Respir. Cell Mol. Biol.* **16,** 187–193.

Sales, N., Dutriez, I., Maziere, B., Ottaviani, M., and Roques, B. P. (1991). Neutral endopeptidase 24.11 in rat peripheral tissues: Comparative localization by '*ex vivo*' and '*in vitro*' autoradiography. *Regul. Pept.* **33,** 209–222.

Scholzen, T. E., and Luger, T. A. (2004). Neutral endopeptidase and angiotensin-converting enzyme—key enzymes terminating the action of neuroendocrine mediators. *Exp. Dermatol.* **13**(Suppl. 4), 22–26.

Scholzen, T. E., Steinhoff, M., Bonaccorsi, P., Klein, R., Amadesi, S., Geppetti, P., Lu, B., Gerard, N. P., Olerud, J. E., Luger, T. A., Bunnett, N. W., Grady, E. F., *et al.* (2001). Neutral endopeptidase terminates substance P-induced inflammation in allergic contact dermatitis. *J. Immunol.* **166,** 1285–1291.

Schweizer, A., Valdenaire, O., Nelböck, P., Deuschle, U., Dumas, M., Edwards, J. B., Stumpf, J. G., and Löffler, B. M. (1997). Human endothelin converting enzyme (ECE-1): Three isoforms with distinct subcellular localizations. *Biochem. J.* **328,** 871–877.

Simonini, G., Azzari, C., Gelli, A. M., Giani, T., Calabri, G. B., Leoncini, G., Del Rosso, A., Generini, S., Cimaz, R., Cerinic, M. M., and Falcini, F. (2005). Neprilysin levels in plasma and synovial fluid of juvenile idiopathic arthritis patients. *Rheumatol. Int.* **25,** 336–340.

Skeggs, L. T., Kahn, J. R., and Shumway, N. P. J. (1956). The preparation and function of the hypertensin-converting enzyme. *Exp. Med.* **103,** 295–299.

Skoog, I., and Gustafson, D. (2006). Update on hypertension and Alzheimer's disease. *Neurol. Res.* **28,** 605–611.

Slomiany, B. L., and Slomiany, A. (2005). Endothelin-1-dependent leptin induction in gastric mucosal inflammatory responses to *Helicobacter pylori* lipopolysaccharide. *Biochem. Biophys. Res. Commun.* **336,** 1106–1111.

Slomiany, B. L., Piotrowski, J., and Slomiany, A. (2000). Up-regulation of endothelin-converting enzyme-1 in gastric mucosal inflammatory responses to *Helicobacter pylori* lipopolysaccharide. *Biochem. Biophys. Res. Commun.* **267,** 801–805.

Snowdon, D. A., Greiner, L. H., Mortimer, J. A., Riley, K. P., Greiner, P. A., and Markesbery, W. R. (1997). Brain infarction and the clinical expression of Alzheimer disease. The Nun Study. *JAMA* **277,** 813–817.

Sparks, D. L., Sabbagh, M., Connor, D., Soares, H., Lopez, J., Stankovic, G., Johnson-Traver, S., Ziolkowski, C., and Browne, P. (2006). Statin therapy in Alzheimer's disease. *Acta Neurol. Scand. Suppl.* **185,** 78–86.

Takahashi, M., Matsushita, Y., Iijima, Y., and Tanzawa, K. (1993). Purification and characterization of endothelin converting enzyme from rat lung. *J. Biol. Chem.* **268,** 21394–21398.

Takahashi, M., Fukuda, K., Shimada, K., Barnes, K., Turner, A. J., Ikeda, M., Koike, M., Yamamoto, Y., and Tanzawa, K. (1995). Localization of rat endothelin converting enzyme to vascular endothelial cells and some secretory cells. *Biochem. J.* **311,** 657–665.

Thomas, J. E., Rylett, C. M., Carhan, A., Bland, N. D., Bingham, R. J., Shirras, A. D., Turner, A. J., and Isaac, R. E. (2005). *Drosophila melanogaster* NEP2 is a new soluble member of the neprilysin family of endopeptidases with implications for reproduction and renal function. *Biochem. J.* **386,** 357–366.

Thornton, E., Vink, R., Blumbergs, P. C., and Van Den Heuvel, C. (2006). Soluble amyloid precursor protein α reduces neuronal injury and improves functional outcome following diffuse traumatic brain injury in rats. *Brain Res.* **1094,** 38–46.

Tikellis, C., Johnston, C. I., Forbes, J. M., Burns, W. C., Burrell, L. M., Risvanis, J., and Cooper, M. E. (2004). Characterization of renal angiotensin-converting enzyme 2 in diabetic nephropathy. *Hypertension* **41,** 392–397.

Tipnis, S. R., Hooper, N. M., Hyde, R., Karran, E., Christie, G., and Turner, A. J. (2000). A human homolog of angiotensin-converting enzyme. Cloning and functional expression as a captopril-insensitive carboxypeptidase. *J. Biol. Chem.* **275,** 33238–33243.

Tran-Paterson, R., Willard, H. F., and Letarte, M. (1989). The common acute lymphoblastic leukemia antigen (neutral endopeptidase-3.4.24.11) gene is located on human chromosome 3. *Cancer Genet. Cytogenet.* **42,** 129–134.

Turner, A. J., and Hooper, N. M. (2002). The angiotensin-converting enzyme gene family: Genomics and pharmacology. *Trends Pharmacol. Sci.* **23,** 177–183.

Turner, A. J., and Tanzawa, K. (1997). Mammalian membrane metallopeptidases: NEP, ECE, KELL, and PEX. *FASEB J.* **11,** 355–364.

Turner, A. J., Barnes, K., Schweizer, A., and Valdenaire, O. (1998). Isoforms of endothelinconverting enzyme: Why and where? *Trends Pharmacol. Sci.* **19,** 483–486.

Turner, A. J., Isaac, R. E., and Coates, D. (2001). The neprilysin (NEP) family of zinc metalloendopeptidases: Genomics and function. *Bioessays* **23,** 261–269.

Turner, A. J., Fisk, L., and Nalivaeva, N. N. (2004). Targeting amyloid-degrading enzymes as therapeutic strategies in neurodegeneration. *Ann. NY Acad. Sci.* **1035,** 1–20.

Turner, P. R., O'Connor, K., Tate, W. P., and Abraham, W. C. (2003). Roles of amyloid precursor protein and its fragments in regulating neural activity, plasticity and memory. *Prog. Neurobiol.* **70,** 1–32.

Usmani, B. A., Harden, B., Maitland, N. J., and Turner, A. J. (2002). Differential expression of neutral endopeptidase-24.11 (neprilysin) and endothelin-converting enzyme in human prostate cancer cell lines. *Clin. Sci. (Lond.)* **103**(Suppl. 48), 314S–317S.

Valdenaire, O., Lepailleur-Enouf, D., Edidy, G., Thouard, A., Barrer, A., Vranckx, R., Tougard, C., and Michel, J. B. (1999). A fourth isoform of endothelin-converting enzyme (ECE-1) is generated from an additional promoter. *Eur. J. Biochem.* **264,** 341–349.

Vassar, R., and Citron, M. (2000). Aβ-generating enzymes: Recent advances in β- and γ-secretase research. *Neuron* **27,** 419–422.

Wen, Y., Onyewuchi, O., Yang, S., Liu, R., and Simpkins, J. W. (2004). Increased β-secretase activity and expression in rats following transient cerebral ischemia. *Brain Res.* **1009,** 1–8.

Wolfe, M. S., Xia, W., Ostaszewski, B. L., Diehl, T. S., Kimberly, W. T., and Selkoe, D. J. (1999). Two transmembrane aspartates in presenilin-1 required for presenilin endoproteolysis and γ-secretase activity. *Nature* **398,** 513–517.

Yasojima, K., Akiyama, H., McGeer, E. G., and McGeer, P. L. (2001). Reduced neprilysin in high plaque areas of Alzheimer brain: A possible relationship to deficient degradation of β-amyloid peptide. *Neurosci. Lett.* **297,** 97–100.

Xu, D., Emoto, N., Giaid, A., Slaughter, C., Kaw, S., de Wit, D., and Yanagisawa, M. (1994). ECE-1: A membrane-bound metalloprotease that catalyzes the proteolytic activation of big endothelin-1. *Cell* **78,** 473–845.

Yu, G., Nishimura, M., Arawaka, S., Levitan, D., Zhang, L., Tandon, A., Song, Y. Q., Rogaeva, E., Chen, F., Kawarai, T., Supala, A., Levesque, L., *et al.* (2000). Nicastrin modulates presenilin-mediated notch/glp-1 signal transduction and APP processing. *Nature* **407,** 48–54.

Zhang, Y., Adner, M., and Cardell, L. O. (2004). Interleukin-1β attenuates endothelin B receptor-mediated airway contractions in a murine *in vitro* model of asthma: Roles of endothelin converting enzyme and mitogen-activated protein kinase pathways. *Clin. Exp. Allergy* **34,** 1480–1487.

Zhu, G., Wang, D., Lin, Y. H., McMahon, T., Koo, E. H., and Messing, R. O. (2001). Protein kinase Cϵ suppresses Aβ production and promotes activation of α-secretase. *Biochem. Biophys. Res. Commun.* **285,** 997–1006.