Published in final edited form as:
J Alzheimers Dis. 2013 ; 33(0 1): S101–S110. doi:10.3233/JAD-2012-129043.

Endothelin-Converting Enzymes and Related Metalloproteases in Alzheimer's Disease

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Abstract

The efficient clearance of amyloid β (Aβ) is essential to modulate levels of the peptide in the brain and to prevent it from accumulating in senile plaques, a hallmark of AD pathology. We and others have shown that failure in Aβ catabolism can produce elevations in Aβ concentration similar to those observed in familial forms of Alzheimer's disease (AD). Based on the available evidence, it remains plausible that in late-onset AD, disturbances in the activity of Aβ degrading enzymes could induce Aβ accumulation, and that this increase could result in AD pathology. The following review presents a historical perspective of the parallel discovery of three vasopeptidases, neprilysin (NEP) and endothelin-converting enzymes-1 and -2 (ECE-1 and ECE-2), as important Aβ degrading enzymes. The recognition of the role of these vasopeptidases in Aβ degradation, beyond bringing to light a possible explanation of how cardiovascular risk factors may influence AD risk, highlights a possible risk of the use of inhibitors of these enzymes for other clinical indications such as hypertension. We will discuss in detail the experiments conducted to assess the impact of vasopeptidase deficiency (through pharmacological inhibition or genetic mutation) on Aβ accumulation, as well as the cooperative effect of multiple Aβ degrading enzymes to regulate concentration of the peptide at multiple sites, both intracellular and extracellular, throughout the brain.

Introduction

Increased levels of amyloid β (Aβ) peptides are among the earliest detectable abnormalities in Alzheimer's disease (AD) and while it is certainly not proven, it is widely accepted that the abnormal accumulation of Aβ in the brain is central to the pathogenesis of the disease. It was 25 years ago when Glenner and colleagues, and subsequently others, identified Aβ as the main component of senile plaques [1]. Their discovery led to the proposal of the amyloid hypothesis which points to Aβ accumulation as the trigger of AD pathology [2]. This hypothesis has been reinforced by the discovery of familial AD (FAD) mutations in the amyloid precursor protein (APP) and in the presenilins, components of the γ-secretase complex [3]. Further, the inexorable development of AD in Down syndrome patients [4] due to an additional copy of the APP gene supported the role of Aβ in the development of AD. In years following the proposal of the amyloid hypothesis, there has been a transition from the original belief that insoluble Aβ deposits were the main pathogenic insult, to the consideration that intermediate soluble amyloid aggregates, known as oligomers, may be the most toxic of the Aβ species [5]. Nevertheless, the accumulation of Aβ is remains a cogent explanation for the cause of AD.

While the identification of the genes involved in FAD contributed to understanding the key players in APP processing and Aβ production, the cause of sporadic AD, however, remains obscure. Several risk factors have been identified that modulate secretase activity *in vitro* and in animal models, but unequivocal proof of dysregulation in APP processing has not been demonstrated in late-onset AD patients. For this reason, we and others began to study

the critical role of Aβ clearance, particularly proteolytic degradation, in regulating Aβ concentration and preventing its accumulation under normal conditions. As is the case with any metabolite, the rate of Aβ accumulation depends as much on its production as on its removal. Removal can occur intracellularly by proteolysis within the very cells producing the peptide, in the extracellular space by secreted or cell-surface proteases, by astrocytes and microglia following phagocytosis, or by clearance across the blood-brain barrier and degradation outside of the central nervous system (CNS). Aβ is rapidly cleared from the brain under normal conditions, with a half life of ~15–30 minutes [6, 7]. Therefore, it is readily apparent how a disturbance in the enzymes and pathways contributing to this efficient clearance could shift Aβ concentration toward a pathogenic level. In 1998, Takaomi Saido first estimated that a 30–50% reduction in Aβ catabolism could produce an accumulation of Aβ to levels similar to those observed in FAD [8]. It is plausible then, that while in FAD there is saturation of Aβ clearance pathways due to higher production of Aβ, a more profound alteration in Aβ removal could underlie sporadic AD.

Discovery of Aβ degrading enzymes in the brain

While the last decade of the twentieth century was remarkable for the characterization of the gene mutations causing FAD and the identification of the enzymes responsible for Aβ generation, the first part of this century has seen a significant increase in our understanding of the Aβ degrading enzymes (ADE) that limit its accumulation in the brain. In a landmark study published in 2000, Iwata et al. infused radiolabeled Aβ42 into the hippocampus of rats and determined the rate of its degradation in the presence or absence of multiple classes of protease inhibitors. This study demonstrated, for the first time *in vivo*, the dramatic effect of proteolytic degradation on the rate of Aβ clearance [9]. Specifically, the metalloprotease inhibitor phosphoramidon and the more selective neprilysin (NEP) inhibitor thiorphan inhibited the degradation of the infused Aβ, leading the authors to propose that NEP was the major ADE in the brain. Soon after, Iwata and colleagues further established the physiological relevance of NEP in Aβ metabolism by reporting increased Aβ40 and Aβ42 accumulation in the brains of NEP knockout mice [10].

Clearly, these initial studies demonstrated the importance of NEP in the degradation of Aβ in the brain. Nonetheless, more recent studies by our group and others show that the proteolytic degradation of Aβ peptides is considerably more complex, and no single protease is responsible for the degradation of all cellular and extracellular pools of Aβ, nor all aggregation states [11]. Parallel to the initial characterization of NEP by Saido's group, we found that phosphoramidon, but not thiorphan, increased the levels of Aβ in cultured neuronal cells without altering APP processing [12]. This observation led us to the discovery of the endothelin-converting enzymes (ECEs) as significant contributors to Aβ catabolism. We found that ECE-1 activity degrades Aβ within the cell, resulting in a net decrease in Aβ secretion and decreased extracellular accumulation. We quickly followed up on the finding that the ECEs degrade Aβ by analyzing Aβ accumulation mice deficient in either ECE-1 or ECE-2, and in 2003 we demonstrated that the levels of both Aβ40 and Aβ42 were significantly elevated in the brains of these knockout mice. Levels of full-length APP and APP C-terminal fragments were unchanged, indicating that ECEs regulate the turnover of Aβ without affecting APP processing. These studies confirmed that both ECE-1 and ECE-2 are physiological regulators of Aβ accumulation in the brain [13].

Alongside the ECEs and NEP, other enzymes have been proposed as ADEs including insulin degrading enzyme (IDE), the plasmin system, angiotensin-converting enzyme (ACE), matrix metalloproteinases, cathepsins, neprilysin-2, and acyl peptide hydrolase [14–16]. Close examination of these ADE candidates reveals that many of these enzymes not only participate in Aβ catabolism but are also well-studied vasopeptidases. ACE, NEP, and

ECE each fall into this category based on their ability to either generate or inactivate vasoactive peptides: ACE generates angiotensin II (Ang-II) and inactivates bradykinin, NEP degrades the natriuretic peptides, bradykinin, and endothelin and ECEs generate endothelins [17].

The Vasopeptidases: ECE-1 and ECE-2, NEP and ACE

During the time that we and others were establishing the ECEs and NEP as ADEs, a new group of drugs aimed at inhibiting vasopeptidase activity was showing promise for the treatment of hypertension. Selective ACE inhibitors had already been in use for well over a decade and these new drug candidates inhibited NEP instead of, or in addition to, ACE. In animal models of hypertension, the dual ACE-NEP inhibitors were shown to be more effective than selective ACE inhibitors [18]. Subsequently, compounds were developed that inhibited NEP and ECE together, or even NEP, ECE, and ACE. These triple inhibitors have also shown promise in animal models [19]. However, based on evidence that Aβ accumulation may begin decades prior to the appearance of clinical symptoms of AD, we became concerned that long-term inhibition of the vasopeptidases NEP and ECE could elevate Aβ levels and possibly promote the development of AD. This potential side effect would not likely be detected during the time-frame of standard clinical trials and demanded careful consideration. Following a report by Hemming et al. [20] in 2005 that ACE, too, could directly degrade Aβ *in vitro*, we intensified our efforts to better understand the consequences of vasopeptidase deficiency on Aβ accumulation in the brain. In the following sections, we will review the functions of the ECEs, NEP, and ACE, discussing evidence for their role in regulating Aβ levels, and rationale leading to our examination of their ability to prevent Aβ accumulation *in vivo*, in our study published in 2006 [11].

Endothelin-converting enzymes

ECEs are members of the M13 family of zinc-metalloproteases; membrane bound endopeptidases that preferentially cleave at the amino side of hydrophobic residues [21]. As suggested by their name, ECEs are characterized by their ability to hydrolyze a family of biologically inactive intermediates, big endothelins (big ET-1,-2 and-3) precisely at the Trp21-Val/Ile22 bond to form ET-1, ET-2 and ET-3 [22]. ETs exert their biological effects through interaction with endothelin receptors, ET_A and ET_B [23, 24], and play a central role in the regulation of blood flow. Vasoconstriction is mediated largely through stimulation of ET_A receptors on smooth muscle cells, and is modulated by the vasodilatory effects of ET_B stimulation on endothelial cells [25–27]. So far, three members of the ECE family have been identified; ECE-1, ECE-2, and ECE-3, each encoded by a different gene. Unlike the other family members, ECE-3 (aka Kell blood group) is mostly found in erythroid tissue and not expressed in the CNS [28]. The other components of the endothelin system work broadly throughout the CNS, playing a major role in neurohormonal homeostasis [29], guiding sympathetic neurons during development [30], and regulating the inflammatory response and monocyte passage through the blood brain barrier [31]. The endothelin receptors have also been shown to mediate the neuronal apoptosis rate in the dentate gyrus during postnatal development and pathology-induced apoptosis [32].

Endothelin-converting enzyme-1

ECE-1 was the first member of the ECE family to be identified [33]. It contains a single transmembrane region flanked by a small amino-terminal cytosolic segment and a carboxy-terminal peptidase domain. The topology of ECE-1 is such that the active site is within the extracellular space or within the lumen of organelles and vesicles, providing access to substrates. There are four human isoforms which only differ in the cytoplasmic tail sequence that defines their subcellular localization [34, 35]. While ECE-1a is mostly found on the

plasma membrane, ECE-1b is exclusively intracellular, with presence within trans-golgi network, endosomes, multivesicular bodies and secretory vesicles. ECE-1d also shows an intracellular location but limited only to endosomes. ECE-1c is located both on the plasma membrane and in intracellular compartments.

The tissue distribution of ECE-1 aligns with its lead role in controlling vascular tone, as it is abundantly expressed in the vascular endothelial cells of all organs. However, ECE-1 is also expressed in non-vascular cells of tissues like brain, lung, pancreas, testis, ovary and adrenal gland [33, 36]. Within the CNS, ECE-1 expression is prominent in diverse nuclei of the hypothalamus including the supraoptic nucleus, the arcuate nucleus (pointing to a function in the neurosecretory system) and other areas, namely the locus coeruleus, substantia nigra, thalamic nuclei, granular layer of the olfactory bulb, red nucleus, raphe nuclei and Purkinje cells in cerebellum. In cortex and hippocampus, ECE-1 is highly expressed in the pyramidal neurons in layer V and throughout all *cornus ammonis*, especially in CA4 [37, 38].

In addition to big ET-1, other substrates of ECE-1 have been identified *in vitro*, including substance P, bradykinin [39], neurotensin, angiotensin I, and somatostatin [21, 40]. Substance P is a neuropeptide important in the physiology of pain and control of neurogenic inflammation, and somatostatin and neurotensin are hypothalamic neuropeptides that reinforce the involvement of ECE activity in neurosecretion. ECE-1 regulatory activity is mediated by a variety of mechanisms. It can occur by degradation of pro-active peptides within the secretory pathway, extracellularly, or by degradation of substrates bound to G protein-coupled receptors in endosomes. ECE endosomal activity regulates receptor recycling, resensitization, and intracellular signaling [41] [42, 43]. Due to the distinct location of ECE-1 isoforms within the cell, some isoforms may favor the hydrolysis of specific substrates.

Several recent reports indicate that genetic variants in *ECE1* may influence risk for developing late-onset AD. A single nucleotide polymorphism, C-338-A, has been identified within the promoter region of ECE-1b that creates a new E2F-2 transcription factor binding site and enhances expression of this intracellular isoform [44, 45]. Several reports indicate that individuals homozygous (AA) for this SNP have reduced risk for developing AD [44, 46, 47], but others report negative findings [48, 49]. Continued evaluation in larger populations is warranted in order to fully understand the role of ECE-1 variants in modulating risk for AD.

Endothelin-converting enzyme-2

ECE-2 is highly homologous and structurally comparable to ECE-1 [50]. It has similar substrate affinities and cleaves big ET-1 with the same catalytic efficiency as ECE-1. However, ECE-2 has a lower pH optimum, of 5–5.5, which strongly suggests that it is active exclusively in intracellular locations [50]. Like ECE-1, ECE-2 cleaves big ET-1 most efficiently among the 3 big ETs, and at least 4 isoforms of human ECE-2 are produced from a single gene [51, 52]. Three isoforms, ECE-2A, ECE-2B-1, and ECE-2B-2, appear to be differentially expressed in brain and peripheral tissues. In bovine tissues, ECE-2A mRNA expression was detected predominantly in liver, kidney, adrenal cortex, ovary, testis, and endothelial cells, while ECE-2B-1 and 2B-2 were more highly expressed in brain (both cerebellum and cortex) and adrenal medulla [53]. Limited RT-PCR studies with human tissues showed that ECE-2A, ECE-2B-1, and ECE-2B-2 were expressed in brain and adrenal gland (B>A) and, to a lesser extent, in lung (A>B) [54]. Unlike ECE-1, none of the cytosolic tail regions of these ECE-2 isoforms contains conserved domains, making it unclear how their different sequences contribute to catalytic activity or enzyme localization. A fourth isoform is predicted to be encoded by an unusual *ece2* transcript, RefSeq NM_174046. This

predicted protein contains a complete S-adenosyl methionine/S-adenosyl homocysteine binding domain followed by a full canonical SAM-methyltransferase domain [52]. It, however, lacks the transmembrane and protease domains of the other ECE-2 isoforms.

ECE-2 immuno-studies have detected the enzyme in Purkinje cells in the cerebellum, in fibers within the glial limitans, astrocytes in the subcortical white matter and in close relation with small vessels, in neuronal processes and cell bodies of pyramidal cells of the neocortex and hippocampus[37, 38]. As in the case of ECE-1, ECE-2 has a neuroendocrine distribution consistent with its ability to process different neuropeptides [55]. However, its restricted intracellular location limits its contribution to the extracellular cleavage of big-ET-1 [50] or other neuropeptides. Nonetheless, behavioral impairments in ECE-2 knockout mice [56] stress the importance of studying ECE-2 function throughout the CNS.

Evidence linking genetic variation in ECE-2 to the development of AD is limited. In fact, to our knowledge, only two studies have been conducted, and with conflicting results. In the first report, an unbiased profile for downregulated genes in AD showed ECE-2 mRNA and protein levels highly decreased [57]. On the other hand, a later study by Palmer et al [58], found ECE-2 levels increased in the cortex from AD patients. In both reports the number of cases was limited and a larger cohort study is needed to address the possible direct role of ECE-2 in AD.

Neprilysin

NEP is also a member of the M13 zinc metalloprotease family and it is predominantly located on the plasma membrane, where it is believed to hydrolyze and therefore terminate the actions of peptide neurotransmitters, including the enkephalins and substance P, in the extracellular space [59–61]. NEP also degrades bradykinin and the natriuretic peptides and as such, it has become a target for cardiovascular disease therapeutics. *In vitro* and *in vivo*, NEP has been shown to hydrolyze Aβ40 and Aβ42 in the brain [9, 62], and its presence inversely correlates with amyloid plaque formation [63, 64]. Furthermore, recent studies have shown that both aged mice and human patients with sporadic AD have decreased levels of NEP in their hippocampus and temporal gyrus, brain regions that are particularly sensitive to amyloid accumulation [63, 65].

Few studies have been published that describe a genetic association between the *NEP* gene and the risk of developing AD. Those available studies have been somewhat contradictory in nature or limited to small isolated AD patient populations. For example, one gene-based association study found an increase in AD susceptibility in Finnish patients with either of two *NEP* polymorphisms [66]. This same group went on to report that individuals with both a *NEP* and *IDE* polymorphism were 3-times more susceptible to AD [67]. However, subsequent researchers could not replicate this *NEP* association within a US Caucasian population, and instead identified an entirely novel polymorphism with association to AD [68].

Angiotensin-converting enzyme

ACE is a key player in the renin-angiotensin system (RAS), which is critical to the regulation of blood pressure and fluid and electrolyte balance (reviewed by Turner and Hooper) [69]. In the classical RAS pathway, renin cleaves angiotensinogen to form Ang I, which itself has little effect on blood pressure. Ang I is further cleaved by ACE to form the potent vasopressor peptide Ang II. ACE also inactivates the vasodilators bradykinin and kalliden, potentiating the vasoconstrictor effect of Ang II production. Due to the dual effect of inhibiting Ang II formation and bradykinin and kalliden degradation, ACE inhibition has become an important therapeutic strategy for the treatment of hypertension

Two isoenzymes of ACE are expressed from a single gene located in chromosome 17, a widely-expressed somatic form and a smaller testis-specific form which both exist as ectoenzymes at the plasma membrane. Secretase cleavage appears to release a soluble and active form of the enzyme into plasma and CSF. ACE has both dipeptidyl dipeptidase activity and endopeptidase activity and *in vitro* studies have shown the enzyme to cleave within Aβ and also convert Aβ42 to Aβ40 [20, 70]. These results have not been replicated *in vivo*, however, and results from our group and others call into question whether ACE cleaves Aβ under physiological conditions [11, 71].

A great deal of attention has been given to establishing a genetic association between ACE and AD [72]. A common insertion/deletion (I/D) polymorphism exists in intron 16 of the *ACE* gene, and the DD genotype has been associated with human longevity [73, 74]. In 1999, Kehoe and colleagues first reported that the II genotype was associated with risk for Alzheimer's disease [75], and this finding was subsequently replicated in some population, but not in others. A 2003 meta-analysis of published data including 21 populations in 18 studies concluded that there was a significant association of the I allele with increased risk of AD [76]. The study also suggested that certain *ACE* haplotypes may modestly influence Aβ42 concentration in the CSF of AD patients. However, a more recent multi-center study [77] and the continually updated Alzgene database (www.Alzgene.com) [72], do not currently support an association between *ACE* haplotypes and late-onset AD.

NEP and ECE, but not ACE, are direct physiological regulators of Aβ concentration in brain: Implications for AD risk and the use of vasopeptidase inhibitors for the treatment of hypertension

As discussed, the major motivation for carrying out our study of the effect of reducing multiple vasopeptidase activities was the common use of ACE inhibitors and the development of novel multi-vasopeptidase inhibitors to treat hypertension. While purified human ACE has been reported to degrade Aβ 1-40 [78] and to convert Aβ42 to Aβ40 [70], prior to our study, the physiological relevance of ACE as an Aβ degrading enzyme in animal models had not been examined. The design of our study allowed us first to determine the effect of ACE deficiency in the brain and periphery, and second to evaluate the effect of simultaneous reductions in NEP and ECE activity, through the use of dual inhibitors and gene knockout models. This design also allowed us to begin to investigate the potential interaction among ADEs. The different cellular and tissue distribution of the ADEs had opened the question of whether there is interaction and compensatory activity among these enzymes, or whether they degrade independent pools of Aβ. If the distinct ADEs cooperate in Aβ removal, the pharmacological inactivation of any one enzyme could be compensated for by the remaining active ADEs. On the other hand, restricted confinement of individual ADE activity to specific pools of Aβ could pose a challenge for the use of vasopeptidase inhibitors, especially if they produce an increase in a particular Aβ pool that associates more directly with cellular toxicity.

ACE deficiency does not alter endogenous Aβ levels in the brains of mice

Endogenous Aβ concentration is significantly elevated in the brains of mice deficient in several ADEs, including NEP, ECE-1, ECE-2, and IDE (reviewed in [79]. Following the initial report that ACE could degrade Aβ *in vitro* [20], we became very interested in analyzing Aβ accumulation in the brains of ACE knockout mice. We established a collaboration with Dr. Kenneth Bernstein at Emory University, who provided us with brain tissue from ACE.8 mice [80]. As ACE knockout mice have physiological defects in the cardiovascular system, reproductive system, and others which complicate the interpretation

of effects on specific organs, Bernstein's group generated a series of mouse models using tissue-specific promoters to express ACE in a restricted manner on an ACE-null background [81]. The model we chose for analyzing Aβ concentration in the brain was generated by targeted homologous recombination, resulting in ACE gene expression under control of the α-myosin promoter. This mouse has ACE expression restricted to the heart, and nearly normal blood pressure [80]. ACE activity is completely absent in the brain. We analyzed the levels of both Aβ40 and Aβ42 in the brains of these mice and found no differences between those lacking ACE activity and those with wild type levels. These results indicate that despite the apparent ability of ACE to cleave Aβ *in vitro*, the enzyme does not regulate levels of Aβ in the brains of mice the way that NEP, ECE-1, ECE-2 and IDE do. While it remains possible that ACE might play a role in regulating Aβ concentration in certain circumstances, these initial results were reassuring and suggested that pharmacologic inhibition of ACE for the treatment of hypertension might be safer than we originally thought, at least in terms of their effect on Aβ concentration.

Combined genetic deficiency in NEP and ECEs results in an additive increase in Aβ accumulation in the brains of mice

Having established that ACE activity does not regulate endogenous Aβ concentration in the brain, we next studied the cooperativity of the remaining vasopeptidases (NEP, ECE-1 and ECE-2) in Aβ removal. As discussed earlier, it had previously been reported by our laboratory and Takaomi Saido's that endogenous Aβ levels were significantly elevated in the brains of mice deficient in each of these enzymes. The increases in Aβ concentration were relatively modest, raising the question of whether ADEs can at least partially compensate for the decreased activity of one another. If NEP and ECEs could compensate for one another, one might expect a larger than additive effect of combined deficiency on Aβ accumulation. To investigate this, we crossed NEP knockout mice with either ECE-2 or ECE-1 knockout mice, and found approximately an additive effect on Aβ accumulation in the brain with each NEP or ECE allele knocked out. These results suggest that NEP and ECEs may degrade independent pools of Aβ, governed by cellular or extracellular location, or anatomical location within the brain. In fact, we may have predicted this result based on earlier studies: NEP is localized to the plasma membrane (active site extracellular) and was shown in the study by Saido's group [9] to significantly regulate the concentration of extracellular (or exogenous) Aβ. ECEs, on the other hand, appear to degrade Aβ primarily within the cell [12].

Pharmacological inhibition of ACE or NEP/ECE: Contrasting effects on Aβ accumulation

Following our analysis of genetic deficiency in ACE, NEP and ECE, we next analyzed the effect of acute inhibition of these enzymes in normal mice by intracerebroventricular (ICV) injection of phosphoramidon, FDA-approved ACE inhibitors, and investigational NEP-ECE inhibitors. Consistent with results in ACE deficient mice, acute inhibition of ACE by either captopril or enalapril failed to alter Aβ levels in the brain. This result was in stark contrast to the effect of phosphoramidon or the more selective ECE inhibitor CGS 35066 [82] both of which produced rapid increases in Aβ concentration. Finally, we determined the effect of commonly used oral ACE inhibitors on both plasma and brain Aβ levels in normal mice. Despite nearly complete inhibition of plasma ACE activity, we detected no alterations in Aβ concentration after administration of these drugs. This, again, was in striking contrast to the effect of ECE and NEP inhibition, which produced elevations in the levels of both Aβ40 and Aβ42. Since all of our measurements were made in brains of young mice, it remains possible that ACE activity could affect Aβ accumulation in aged brains or in the case of AD,

where there may be a failure in other Aβ clearance pathways. However, our finding that ACE inhibitors do not increase Aβ levels in the brain has been independently confirmed in more recent studies of prolonged administration in AD mouse models [71, 83, 84].

The results of these animal studies, together with epidemiological studies showing no increased risk of AD in individuals treated with ACE inhibitors [85, 86], is reassuring. Therefore, from the perspective of AD, ACE could be considered the best therapeutic target among all vasopeptidases. However, the development of drugs that inhibit NEP and ECE instead of, or in combination with ACE, remain very concerning if the amyloid hypothesis is correct. Our studies demonstrate that upon administration of selective ECE inhibitors or combined NEP and ECE inhibitors, CNS and circulating Aβ levels can increase up to 3 fold in a matter of minutes. Apparently abandoned by pharmaceutical companies in the United States, a recent publication indicates that the development of dual NEP-ECE inhibitors is still being pursued in Europe for the treatment of hypertension. It will be very important to test these compounds in long-term studies in animals, as well as to monitor Aβ levels in trial participants. The results of a human research trial have now been published [87] with no mention of the risk of this potential side effect.

Conclusion

Our research has brought to light the important role of ECEs in Aβ degradation. As discussed, ECE dysfunction may produce increases in Aβ concentration similar to those seen in models of familial AD. But beyond ECE metabolism, our line of work and that of others characterizing NEP and IDE demonstrate an important and often overlooked mechanism of Aβ regulation independent of secretase activity. With only a fraction of AD cases currently attributable to known risk factors other than age, it is quite possible that distinct subgroups of AD patients exist for whom decreased Aβ clearance is the precipitating factor for the disease. Many factors, including genetic mutation, transcriptional alteration, and even pharmacological manipulation may decrease the activity of ADEs in the brain. Moreover, based on the lack of compensatory mechanisms among ADEs, decreased activity of each of the ADEs may represent an independent significant risk factor for the development of AD. Although we acknowledge the challenge of isolating and testing the specific activity of each ADE, a re-examination of known risk factors associated with AD from this perspective could prove insightful.

In future studies, particular attention should be focused on the interplay between vascular dysfunctions and AD [88]. Common cardiovascular risk factors like hypercholesterolemia, diabetes, obesity, hyperhomocysteinemia, high blood pressure, or ApoE4 are also risk factors for AD. For some of these risk factors, the contribution appears at mid-life [89–92] before any pathological sign of AD, suggesting a direct effect on the initiation of the disease. Also, often times, AD is accompanied by vascular pathologies attributed to ischemic insults that lower the threshold for the manifestation of AD symptoms. If Aβ accumulation in AD is indeed partially driven by vaosopeptidase dysfunction, alterations in vasopeptide homeostasis may also occur, and may be expected to influence the development of other pathologies. As we have come to acknowledge that Aβ accumulation alone cannot fully account for the pathogenesis of AD, altered levels of certain vasopeptidases may represent additional contributing factors to neuronal toxicity. At the same time, if dysregulations in vasopeptidase acivity are driven by cardiovascular risk factors, more effective therapeutic strategies could be designed.

Acknowledgments

This research was supported by grant numbers NS073512, NS042192, and NS048554 from the NINDS/NIH. Its contents are solely the responsibility of the authors and do not necessarily represent the official views of the NINDS or NIH.

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