SAGE-Hindawi Access to Research
International Journal of Alzheimer's Disease
Volume 2011, Article ID 857368, 7 pages
doi:10.4061/2011/857368

Review Article

Protein Kinase C-Regulated Aβ Production and Clearance

Taehyun Kim, David J. Hinton, and Doo-Sup Choi

Department of Molecular Pharmacology and Experimental Therapeutics, Mayo Clinic College of Medicine, Rochester, MN 55905, USA

Correspondence should be addressed to Doo-Sup Choi, choids@mayo.edu

Received 13 October 2010; Revised 3 December 2010; Accepted 13 December 2010

Academic Editor: Katsuhiko Yanagisawa

Alzheimer's disease (AD) is the most common form of dementia among the elderly population. AD, which is characterized as a disease of cognitive deficits, is mainly associated with an increase of amyloid β-peptide (Aβ) in the brain. A growing body of recent studies suggests that protein kinase C (PKC) promotes the production of the secretory form of amyloid precursor protein (sAPPα) via the activation of α-secretase activity, which reduces the accumulation of pathogenic Aβ levels in the brain. Moreover, activation of PKCα and mitogen-activated protein kinase (MAPK) is known to increase sAPPα. A novel type of PKC, PKCε, activates the Aβ degrading activity of endothelin converting enzyme type 1 (ECE-1), which might be mediated *via* the MAPK pathway as well. Furthermore, dysregulation of PKC-MAPK signaling is known to increase Aβ levels in the brain, which results in AD phenotypes. Here, we discuss roles of PKC in Aβ production and clearance and its implication in AD.

1. Introduction

Alzheimer's disease (AD) is the most common form of dementia among the elderly population [1, 2]. A major hallmark of AD is the abnormal processing and accumulation of neurite plaques containing amyloid β-peptide (Aβ) in the brain [3, 4]. Amyloid precursor protein (APP) is mainly cleaved by the α-secretase enzyme (Figure 1), producing the secretory form of amyloid precursor protein (sAPP; β-amyloid (Aβ) 17–42), which is soluble and nontoxic [5]. However, when APP is cleaved by β- and γ-secretase enzymes [6], it leads to the formation of Aβ1–40 and Aβ1–42, which are insoluble unlike sAPP, and results in the accumulation of amyloid plaques [7]. In the production of Aβ1–42, the Aβ1–42/Aβ1–40 ratio is associated with the amount of insoluble Aβ aggregation [8]. On the other hand, the abnormal hyperphosphorylation of tau results in insoluble fibrils and neurofibillary tangels in the brain [9, 10]. Thus, an understanding of the pathological processes of APP and tau in AD is a critical therapeutic target in preventing or delaying AD in humans [11–13]. Here, we review the role of protein kinase C (PKC) in Aβ production and clearance through α-secretase or Aβ-degrading enzyme activity. Among several PKCs, we focus on the role of PKCε in Aβ levels because several recent findings have demonstrated that the activation or overexpression of PKCε promotes the Aβ degradation activity of endothelin converting enzyme type 1 (ECE-1) [14, 15].

2. PKC and Aβ Plaques

PKC is a phospholipid-dependent serine/threonine kinase and consists of at least 12 isoenzymes [18, 19]. PKCs can be classified into three subfamilies based on their protein structure and second messenger requirements: conventional (or classical), novel, and atypical. Conventional PKCs contain the α, β1, β2, and γ isoforms and require Ca^{2+}, diacylglycerol (DAG), and a phospholipid such as phosphatidylcholine for activation. Novel PKCs include the δ, ε, η, θ, and μ isoforms and require DAG or phospholipids but do not require Ca^{2+} for activation. On the other hand, atypical PKCs consisting of protein kinase ζ, ι, and λ isoforms do not require either Ca^{2+} or diacylglycerol for activation [20].

Numerous studies have suggested that phorbol 12-myristate 13-acetate (PMA), a nonspecific PKC activator, is capable of lowering secreted Aβ levels in neurons [21–24]. Based on these results, several studies have attempted to identify precisely which PKC isozyme actually regulates

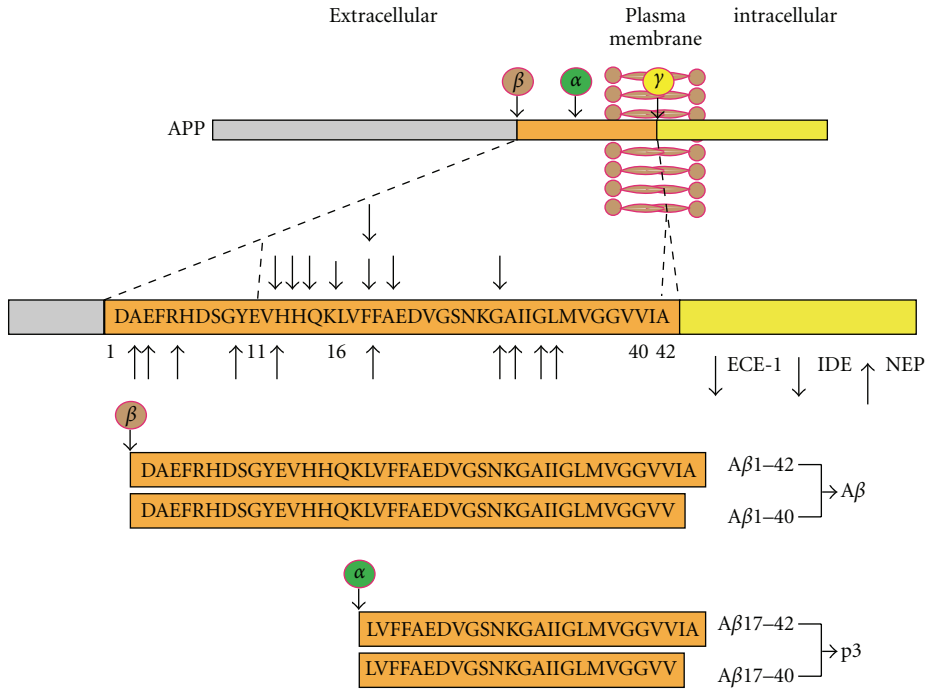

Figure 1: Amyloid metabolism by secretases and Aβ-degradation enzymes (ECE-1, IDE, NEP). Aβ-degrading proteases play an important role in regulating Aβ levels via known cleavage sites (adapted from [1, 16, 17]).

APP processing. The overexpression of PKCα or PKCε, but not PKCθ, has been shown to induce APP secretion from cells [25]. Interestingly, specific inhibition of either PKCα or PKCθ in CHO cells expressing APP695 was associated with a loss of PMA-mediated APP secretion [26]. In addition, experiments with a dominant negative fragment of PKCε reduced phorbol ester-induced secretion of sAPPα [15, 27]. However, even though intraparenchymal administration of phorbol esters reduces Aβ levels and decreases amyloid plaque density in mice expressing an amyloidogenic variant of human APP, α-secretase activity is not increased in the brain [28]. This raises the possibility that PKC reduces Aβ levels *in vivo* by another mechanism.

3. Aβ Clearance and Peptidases

The accumulation of Aβ in the brain is one of the main symptoms of AD [3]. An abnormality in the proteolytic degradation of Aβ appears to be associated with the progression of AD [29]. As shown in Figure 1, several proteases that degrade Aβ in mice include insulin-degrading enzyme (IDE), neprilysin (NEP), and endothelin-converting enzyme (ECE) 1 and 2 [16, 30]. IDE (insulysin) is a ~ 110 KDa thiol zinc-metalloendopeptidase which is expressed in the cytosol, peroxisomes, and endosomes and on cell surfaces, and it is the major enzyme responsible for insulin degradation *in vitro* [31]. However, IDE has also been found to degrade Aβ in neuronal and microglial cells [32] and to eliminate the neurotoxic effects of Aβ [33]. Consistently, IDE-null mice showed increased levels of Aβ in the brain [34]. NEP is another key player in Aβ clearance [35]. In the brain, NEP is mainly expressed on neuronal plasma membranes [36]. NEP-null mice show defects in both the degradation of exogenously administered Aβ and in the metabolic suppression of endogenous Aβ levels in a gene dose-dependent manner [37]. The importance of these zinc-metalloendopeptidases in Aβ clearance is demonstrated by the fact that the transgenic overexpression of IDE or NEP in neurons significantly reduces Aβ levels and plaque associated with AD pathology [38]. Angiotensin-converting enzyme (ACE) is a membrane-bound zinc metalloprotease [39]. ACE mainly converts angiotensin I to angiotensin II, which is critical in the regulation of blood pressure, body fluid, and sodium homeostasis [40]. Recent studies indicate that ACE expression also promotes the degradation of Aβ [41].

Several receptor-mediated Aβ clearance mechanisms have already been examined [42]. Low-density lipoprotein receptor-related protein (LRP) and the receptor for advanced glycation end products (RAGE) regulate Aβ levels across the blood-brain barrier [43]. Both LRP and RAGE are multiligand cell surface receptors that mediate the clearance of a large number of proteins in addition to Aβ. LRP mainly removes Aβ from the brain to the periphery whereas RAGE appears to influx Aβ back to the brain from the periphery [42, 43].

4. Endothelin-Converting Enzymes (ECEs)

ECEs are a class of type II transmembrane metalloproteases, which convert pro-ET into endothelin [44]. Two different ECEs, including ECE-1 and ECE-2, are expressed in brain regions related to AD [45, 46]. Although ECE-1 is

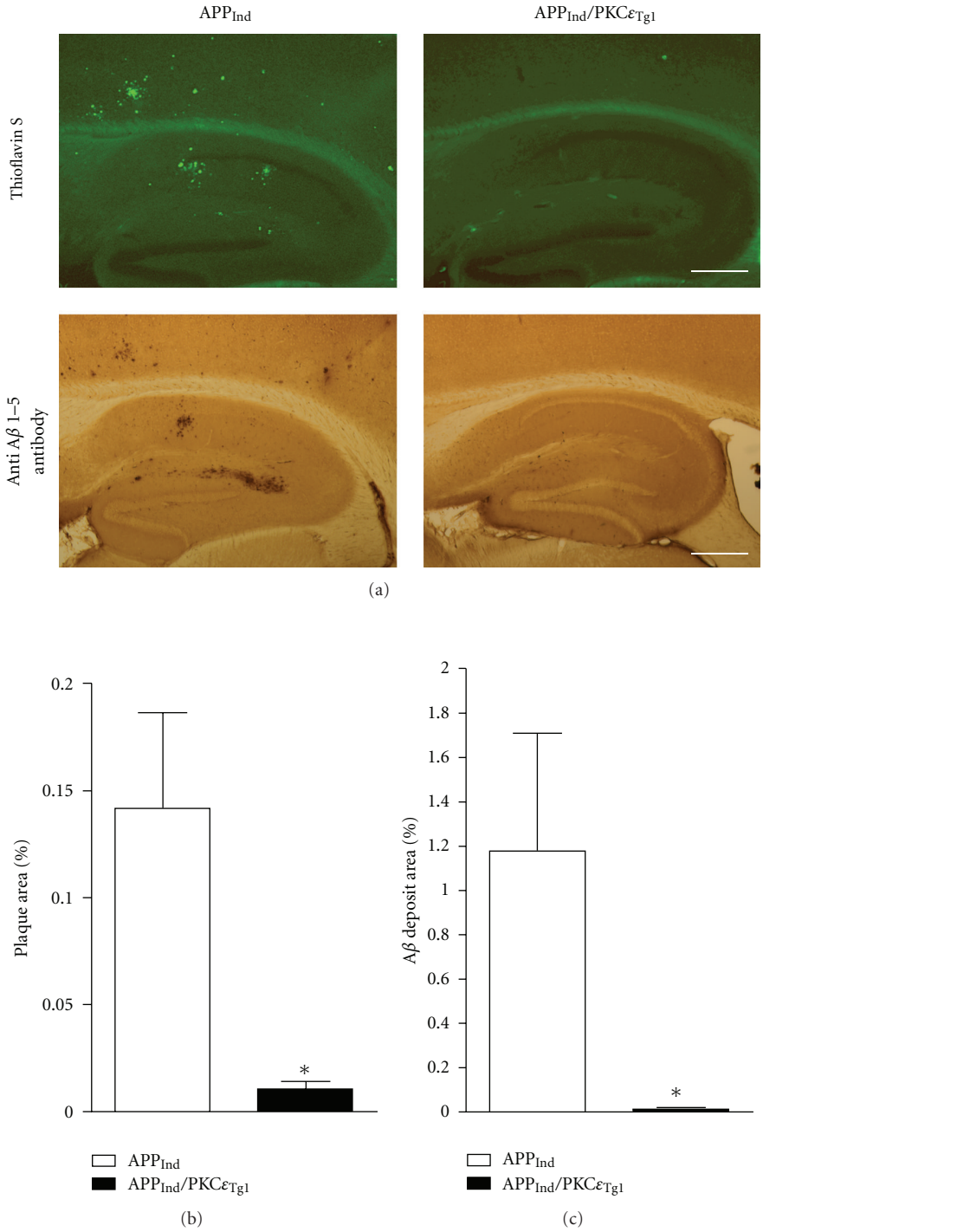

FIGURE 2: Overexpression of PKCε reduces the amyloid plaque burden and inhibits Aβ accumulation in brain parenchyma. (a) Thioflavin S staining and anti-Aβ immunostaining revealed fewer plaques and Aβ immunoreactive deposits in the hippocampus and neocortex in APP$_{Ind}$/PKCε_{Tg1} mice than in APP$_{Ind}$ mice. Scale bar: 200 μm. Quantification of (b) thioflavin S staining and (c) Aβ deposits in hippocampus and cortex sections (adapted from [14]). $^*P < .05$ by two-tailed t-test.

abundantly expressed in vascular endothelial cells [47], it is also expressed in nonvascular cells, including hippocampal and neocortical pyramidal neurons, cerebellar Purkinje cells, and astrocytes [48]. ECE-2 is also expressed in the brain, especially in several subpopulations of neurons in the thalamus, hypothalamus, amygdala, and hippocampus [46]. Studies have demonstrated that ECE-1 is a key enzyme for the degradation of Aβ in the brain [49]. The *in vivo* function of ECE has been examined in ECE-1 heterozygous (+/−) and ECE-2 null (−/−) mice. In both cases, levels of Aβ were

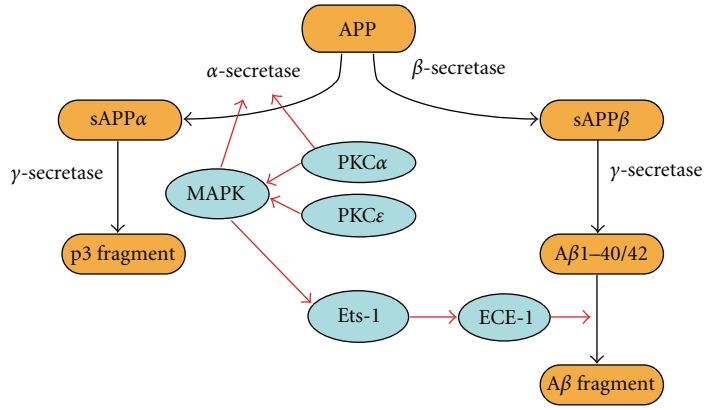

Figure 3: Schematic summary of role of PKC-MAPK-dependent Aβ production and clearance. PKCα upregulates α-secretase activity while PKCε stimulates Aβ-degrading activity of ECE-1, probably via MAPK-dependent Ets-1 pathway. MAPK is also known to activate α-secretase activity independently or through PKC activation.

increased compared with wild-type mice, suggesting that these ECEs are an important Aβ-degrading enzyme *in vivo* [50]. Another study demonstrated that NEP (−/−)/ECE-1 (+/−) or NEP (−/−)/ECE-2 (−/−) mice have increased accumulation of both Aβ1–40 and Aβ1–42 in the brain [51]. Interestingly, a genetic variant of human ECE-1 (ECE1B C-338A) with increased promoter activity was associated with a reduced risk of sporadic AD in a French Caucasian population [45]. ECE-1 degrades synthetic Aβ levels *in vitro* [50] and is the main ECE for Aβ degradation. Recently, the expression of ECE-2 has also been shown to be a relevant Aβ-degrading enzyme and is dramatically increased at both mRNA and protein levels of patients with AD [52].

Endothelin-1 (ET-1) is the major peptide formed by ECE-1, and its cellular actions are mediated via two G-protein coupled receptors, ET_A and ET_B, which are widely distributed in the brain [53]. ET-1 levels appear elevated in postmortem brains from patients with Alzheimer-type dementia [54]. A study indicates that ET-1 is increased in brain microvessels isolated from patients with AD and promotes the survival of brain neurons [55]. However, this effect might be associated with the protective actions of ET-1 *in vivo*, rather than contributing to the AD pathology [56].

5. PKCε, MAPK, and ETS Pathways

The activation of PKCs has suggested a neuroprotective function in animals [57]. PKC activators can also prevent the production of Aβ and extend the survival of AD transgenic mice [58]. However, chronic treatment of nonspecific PKC activators such as phorbol esters at high doses could increase levels of Aβ by decreasing PKC function or increasing APP synthesis [59]. These studies also suggest that the chronic application of phorbol esters may differentially regulate the function of PKC isoforms, downregulating PKCα and upregulating PKCε. There are several mechanisms by which the activation of PKCs could regulate the reduction of Aβ. Interestingly, our recent study demonstrates that overexpression of human PKCε reduces Aβ levels significantly in the brain (Figure 2). As shown in Figure 3, activation of PKCs including PKCα is known to promote α-secretase activity [25, 60], while activation or overexpression of PKCε stimulates Aβ-degrading activity of ECE-1, probably via MAPK-dependent Ets-1 pathway [14, 15]. MAPK is also known to activate α-secretase activity independently [61] or through PKC activation [62–64]. Since MAPK can activate Ets-1 and 2 [65], it is possible that PKCε-mediated MAPK could control ETS pathways and thus regulate ECE expression in the brain. Additionally, ETS transcription factors play a key role in cell growth, differentiation, and survival [66]. ETS proteins form complexes and act synergistically with other transcription factor families such as PEA3 or AP-1 [67]. Ets-1 has been known to be involved in angiogenesis [68]. However, another research indicates that upregulation of Ets-2 is closely associated with AD neurodegenerative lesions in the brain [69].

6. Conclusion

In Alzheimer's disease (AD), it has long been known that activated PKCs reduce Aβ levels in the brain. PKC is also suggested to be a functional biomarker of AD [70]. The steady-state level of Aβ depends on a balance between production and clearance. In addition to Aβ production, several researchers suggest that enzyme-mediated degradation of Aβ is also critical for the regulation of Aβ levels [71]. Especially, since PKC is a key modulator in Aβ production or clearance in the brain [15, 58, 72], regulation of PKC activity could be a useful treatment target for AD [14, 73, 74]. However, the functional relevance of each PKC isoform in regulating Aβ levels in AD remains to be studied. Moreover, while α-secretase-mediated cleavage of APP via PKC isoforms reduces amyloid, detailed mechanisms of how PKC isoforms activate the enzyme-degradation system await further investigation. Therefore, PKC isoform-specific ligands or viral-mediated overexpression of PKC isoform as well as specific shRNAs approaches may unveil detailed molecular bases that underlie PKC-regulated Aβ clearance.

Acknowledgments

The authors thank D. Frederixon for her help in preparing the paper. This research was supported by the Samuel Johnson Foundation for Genomics of Addiction Program at Mayo Clinic, Rochester (DSC).

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