

Review Article

Protein Kinase C-Regulated A β Production and Clearance

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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 (A β) 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 (sAPP α) via the activation of α -secretase activity, which reduces the accumulation of pathogenic A β levels in the brain. Moreover, activation of PKC α and mitogen-activated protein kinase (MAPK) is known to increase sAPP α . A novel type of PKC, PKC ϵ , activates the A β 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 A β levels in the brain, which results in AD phenotypes. Here, we discuss roles of PKC in A β 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 (A β) 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 (A β) 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 A β 1–40 and A β 1–42, which are insoluble unlike sAPP, and results in the accumulation of amyloid plaques [7]. In the production of A β 1–42, the A β 1–42/A β 1–40 ratio is associated with the amount of insoluble A β aggregation [8]. On the other hand, the abnormal hyperphosphorylation of tau results in insoluble fibrils and neurofibrillary tangles 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 A β production and clearance through α -secretase or A β -degrading enzyme activity. Among several PKCs, we focus on the role of PKC ϵ in A β levels because

several recent findings have demonstrated that the activation or overexpression of PKC ϵ promotes the A β degradation activity of endothelin converting enzyme type 1 (ECE-1) [14, 15].

2. PKC and A β 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 , β 1, β 2, and γ isoforms and require Ca²⁺, 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 Ca²⁺ for activation. On the other hand, atypical PKCs consisting of protein kinase ζ , ι , and λ isoforms do not require either Ca²⁺ 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 A β levels in neurons [21–24]. Based on these results, several studies have attempted to identify precisely which PKC isozyme actually regulates

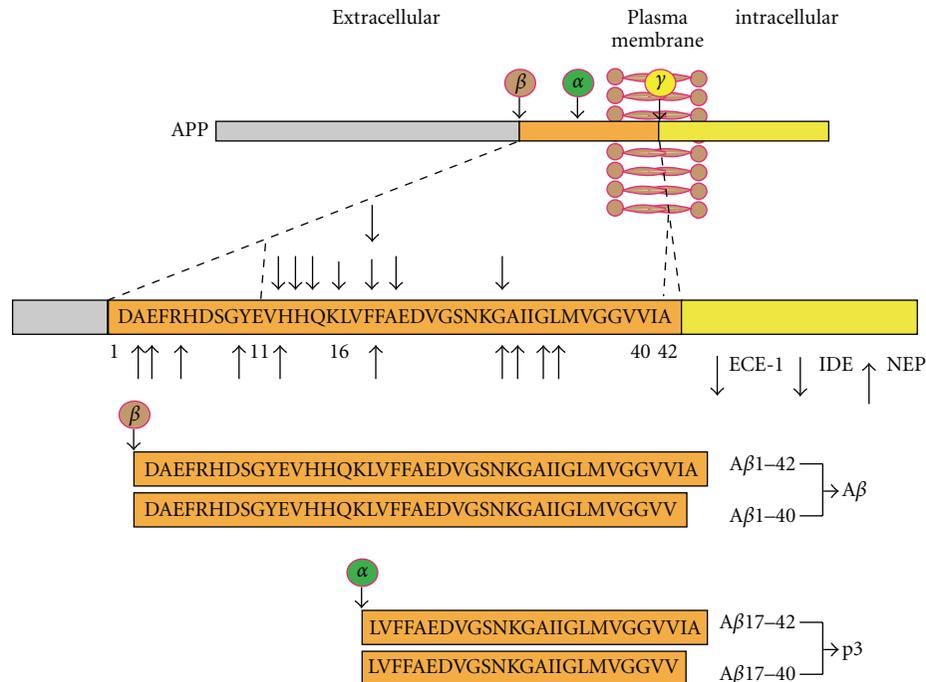


FIGURE 1: Amyloid metabolism by secretases and $A\beta$ -degradation enzymes (ECE-1, IDE, NEP). $A\beta$ -degrading proteases play an important role in regulating $A\beta$ levels via known cleavage sites (adapted from [1, 16, 17]).

APP processing. The overexpression of $PKC\alpha$ or $PKC\epsilon$, but not $PKC\theta$, has been shown to induce APP secretion from cells [25]. Interestingly, specific inhibition of either $PKC\alpha$ or $PKC\theta$ 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 $PKC\epsilon$ reduced phorbol ester-induced secretion of sAPP α [15, 27]. However, even though intraparenchymal administration of phorbol esters reduces $A\beta$ 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 $A\beta$ levels *in vivo* by another mechanism.

3. $A\beta$ Clearance and Peptidases

The accumulation of $A\beta$ in the brain is one of the main symptoms of AD [3]. An abnormality in the proteolytic degradation of $A\beta$ appears to be associated with the progression of AD [29]. As shown in Figure 1, several proteases that degrade $A\beta$ 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 $A\beta$ in neuronal and microglial cells [32] and to eliminate the neurotoxic effects of $A\beta$ [33]. Consistently, IDE-null mice showed increased levels of $A\beta$ in the brain [34]. NEP is another key player in $A\beta$ 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 $A\beta$ and in the metabolic suppression of endogenous $A\beta$ levels in a gene dose-dependent manner [37]. The importance of these zinc-metalloendopeptidases in $A\beta$ clearance is demonstrated by the fact that the transgenic overexpression of IDE or NEP in neurons significantly reduces $A\beta$ 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 $A\beta$ [41].

Several receptor-mediated $A\beta$ clearance mechanisms have already been examined [42]. Low-density lipoprotein receptor-related protein (LRP) and the receptor for advanced glycation end products (RAGE) regulate $A\beta$ 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 $A\beta$. LRP mainly removes $A\beta$ from the brain to the periphery whereas RAGE appears to influx $A\beta$ 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

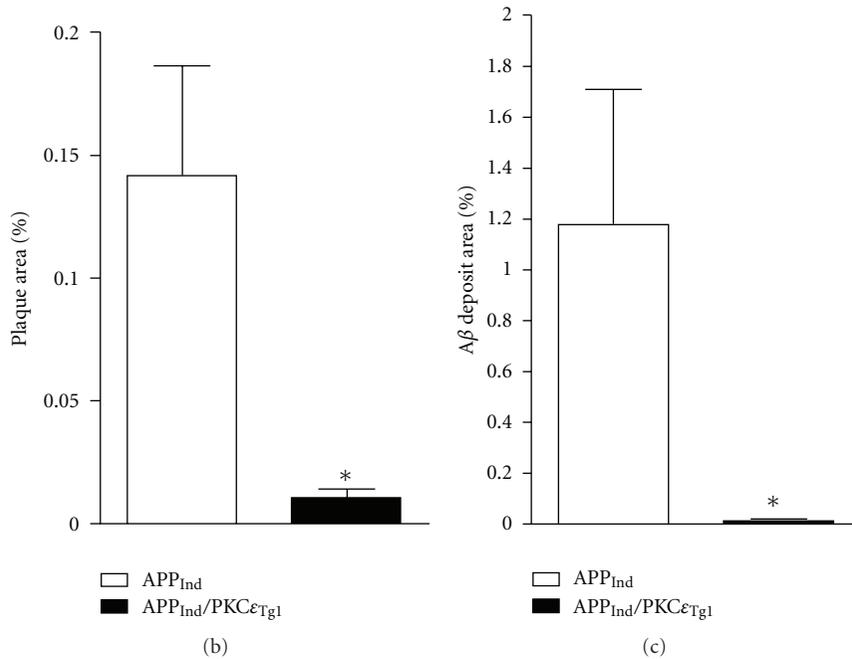
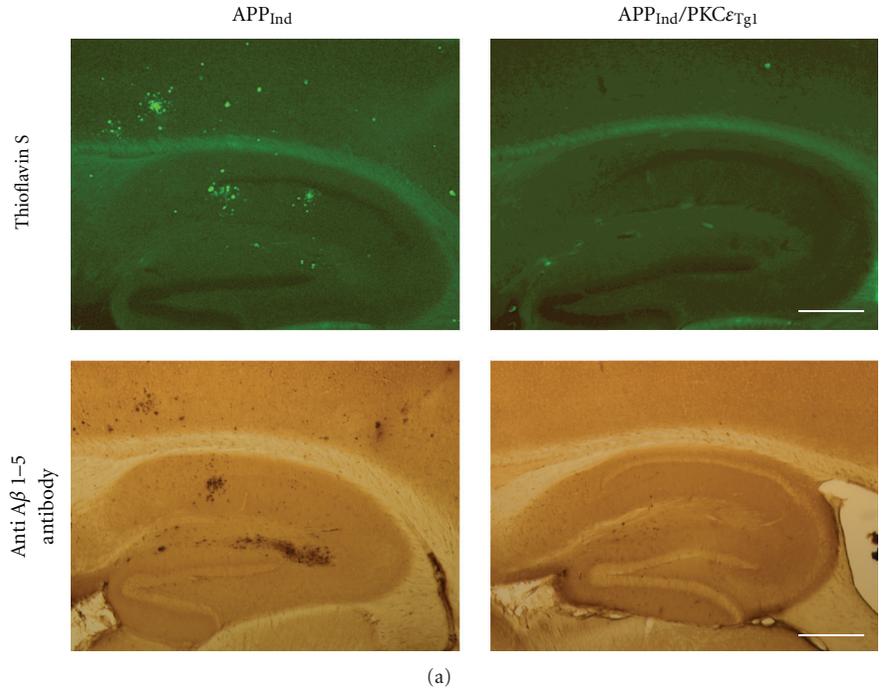


FIGURE 2: Overexpression of PKCε reduces the amyloid plaque burden and inhibits Aβ accumulation in brain parenchyma. (a) Thioflavin S staining and anti-Aβ immunostaining revealed fewer plaques and Aβ immunoreactive deposits in the hippocampus and neocortex in APP_{Ind}/PKCε_{Tg1} mice than in APP_{Ind} 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

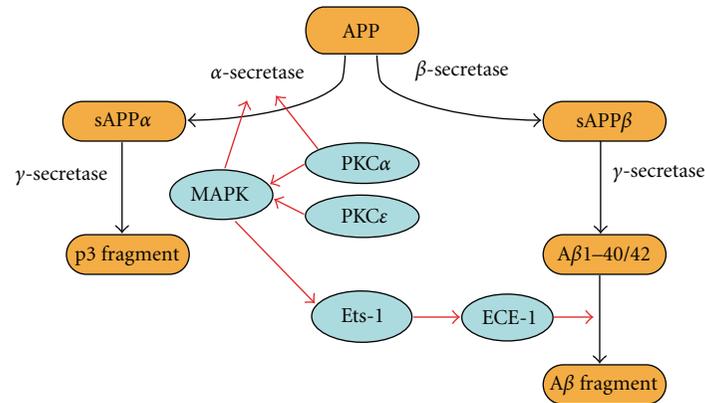


FIGURE 3: Schematic summary of role of PKC-MAPK-dependent $A\beta$ production and clearance. $PKC\alpha$ upregulates α -secretase activity while $PKC\epsilon$ stimulates $A\beta$ -degrading activity of ECE-1, probably via MAPK-dependent Ets-1 pathway. MAPK is also known to activate α -secretase activity independently or through PKC activation.

increased compared with wild-type mice, suggesting that these ECEs are an important $A\beta$ -degrading enzyme *in vivo* [50]. Another study demonstrated that NEP (-/-)/ECE-1 (+/-) or NEP (-/-)/ECE-2 (-/-) mice have increased accumulation of both $A\beta_{1-40}$ and $A\beta_{1-42}$ 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 $A\beta$ levels *in vitro* [50] and is the main ECE for $A\beta$ degradation. Recently, the expression of ECE-2 has also been shown to be a relevant $A\beta$ -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. $PKC\epsilon$, MAPK, and ETS Pathways

The activation of PKCs has suggested a neuroprotective function in animals [57]. PKC activators can also prevent the production of $A\beta$ 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 $A\beta$ 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 $PKC\alpha$ and upregulating $PKC\epsilon$. There are several mechanisms by which the activation of PKCs could regulate the reduction of $A\beta$. Interestingly, our recent study demonstrates that overexpression of human $PKC\epsilon$ reduces $A\beta$ levels significantly in the

brain (Figure 2). As shown in Figure 3, activation of PKCs including $PKC\alpha$ is known to promote α -secretase activity [25, 60], while activation or overexpression of $PKC\epsilon$ stimulates $A\beta$ -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 $PKC\epsilon$ -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 $A\beta$ levels in the brain. PKC is also suggested to be a functional biomarker of AD [70]. The steady-state level of $A\beta$ depends on a balance between production and clearance. In addition to $A\beta$ production, several researchers suggest that enzyme-mediated degradation of $A\beta$ is also critical for the regulation of $A\beta$ levels [71]. Especially, since PKC is a key modulator in $A\beta$ 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 $A\beta$ 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 $A\beta$ clearance.

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