Amyloid Pathology and Protein Kinase C (PKC): Possible Therapeutics Effects of PKC Activators

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Abstract. Amyloid β-protein (Aβ) is one of the most studied peptides in human neurodegenerative disorders. Although much has been learned about the biochemistry of this peptide, fundamental questions such as when and how the Aβ becomes pathologic remain unanswered. In this article we review the recent findings on the biology and pathology of Aβ and the role protein kinase C (PKC) plays in these processes. The potential neuroprotective role of PKC and the possible therapeutic effects of PKC activators in Alzheimer’s disease (AD) will be discussed. Briefly, comments will be also addressed on the role of PKC in cell death and neurogenesis in AD.

Keywords: amyloid protein, Alzheimer’s disease, signal transduction, protein kinase C, cell death

Invited article

Amyloid β-protein localization: normal and pathologic

Amyloid β-protein (Aβ) has been identified initially as a ‘pathologic’ protein associated with the Alzheimer’s dementia. Over the past two decades, extensive studies on the pathogenesis of Aβ have revealed that generation of soluble Aβ is a normal event. Evidence to date demonstrates that the protein is secreted by cells in normal conditions mainly as the 40 amino-acid fragment of Aβ (for review, see ref. 1). Aβ is present in the cerebrospinal fluid and plasma of normal subjects (for review, see ref. 1), but low and detectable amounts of Aβ40 can also be found in cortical tissue of young non-demented individuals (2). In contrast, in normal aged individuals (3) and in a group of elderly non-demented subjects, higher levels of insoluble Aβ42 are reported (2). These findings suggest that although Aβ is secreted throughout life, it begins to accumulate in old age. At the later stage of life, age-dependent biochemical alterations in concert with Alzheimer’s disease (AD) risk factors could accelerate the deposition of readily aggregated Aβ42. In light of these observations, the amyloid hypothesis is currently being reconsidered and again supports a central role of Aβ in the pathophysiology of the AD.

It is now understood that neurons are the main source of Aβ in AD, although it has been reported that astroglia and microglia might synthesize or transport the soluble forms of Aβ from the interstitial space into the senile plaques or vascular walls (see ref. 2). However, the origin of the amyloid deposits identifiable in the extracellular parenchyma in AD is still debated. It has been hypothesized that Aβ gradually accumulates in the extracellular space due to excess secretion and/or deficient clearance (see ref. 2). It has also been suspected that Aβ initially aggregates and accumulates in the neuronal cell body and the cell deposits only become extracellular after the aggregates disrupt the cell integrity. In favor of the latter hypothesis, recent data have revealed the presence of aggregated Aβ42 within neuronal cell bodies by using specific antibodies against
the C-terminus of the Aβ42 molecule (4). The authors have also detected Aβ-like plaque staining that has neuronal shape, suggesting a neuronal origin of amyloid plaques (4). In addition, a new report convincingly describes intracellular Aβ42 immunolabeling in pyramidal neurons and discusses the technical artifacts in the current methods used to detect the amyloid plaques (5). Together these findings support the hypothesis that the extracellular amyloid plaques originate from the intraneuronal pool of accumulated Aβ42. If this is the case, one might suggest that the neuronal aggregation of Aβ42 is an initial and necessary event in the generation of plaques in AD. What triggers the aggregation and deposition of Aβ still remains unclear. Higher prevalence of sporadic rather than genetically-linked cases of AD suggests a random biochemical mechanism that leads to this process. Clinical data together with immunohistochemical analysis of plaques suggested a role for protein kinase C (PKC) in the pathogenesis of Aβ. Namely, previous studies have shown that both diffuse and mature plaques are immunoreactive for the isoform βII of PKC but not for PKC (α), PKC (βI), or PKC (γ), suggesting an early role of PKC (βII) in Aβ aggregation (for review, see ref. 6). Additional studies have shown that anti-PKC (βII) immunostaining is elevated in the diffuse plaques and more pronounced in mature plaques, while very low anti-PKC (βII) reactivity is found outside the deposits. Although, the high intensity of anti-PKC (βII) immunolabeling does not necessarily indicate high activity, the results are compatible with the hypothesis that neuronal hyperactivation at early stages may be involved in AD neuropathology (for review, see ref. 6).

Role of PKC in Aβ generation

PKC is a phospholipid-dependent protein kinase that plays a crucial role in various cellular functions in neuronal and non-neuronal cells. Molecular cloning has identified eleven PKC isoforms with individual characteristics and enriched distribution in the brain (7). In neurons, PKC is a key enzyme in neurotransmission, synaptic plasticity, and learning and memory. Recent in vitro data suggest that certain PKC isoforms are also intimately involved in cell survival (8). With regard to AD, PKC is linked to amyloid precursor protein (APP) processing, regulates the expression of APP mRNA, and as suggested by recent studies, may be involved in presenilin (PS) protein function as well (9).

Aβ is generated from APP, an integral membrane protein ubiquitously expressed in cells. APP is particularly abundant in neurons, but may also be present in astrocytes, microglia, and endothelial smooth muscle. APP can be cleaved at three internal sites by three distinct proteolytic enzymes: α-, β-, and γ-secretases (for review, see ref. 1). Cleavage by β- and γ-secretases at the N and C terminus of APP generates the amyloidogenic fragments of Aβ. The α-pathway is a non-amyloidogenic cascade that is controlled by PKC. α-Secretase enzyme cleaves APP at a site within the Aβ sequence and generates an extracellular soluble fragment (sAPPα) and an intracellular fragment C83, thus precluding the formation of an intact Aβ. These cleavages occur both in brain and in peripheral tissues in AD fibroblasts (10) and could take place in different subcellular organelles (for review, see ref. 11). In vitro studies have demonstrated that different PKC isoforms play distinct roles in APP processing. PKCα is specifically involved in phorbol ester-induced sAPPα release, while PKCε is involved in coupling cholinergic receptors with APP metabolism (12). In vivo studies using a guinea-pig model with constitutive overactivation of PKCα and β isoforms have shown an increase in sAPPα production, indicating that these isoforms are key regulators of α-secretory APP processing (13). Despite of sAPPα increase in this animal model, additional studies have found no changes in tissue concentration of Aβ peptides in the neocortex or hippocampus, suggesting that generation of Aβ through the β-secretase pathway is independently regulated from the α-secretase pathway (14). These findings further suggest that it is questionable to use drug therapies based on PKC activators to reduce Aβ level because increased secretory APP processing does not necessarily affect Aβ generation or slow amyloid plaque formation. In contrast, other lines of evidence obtained in the mouse model have suggested that PKC activators might enhance secretion of APPα and reduce Aβ40 in the brains of APP[V717I] transgenic mice that overexpress the APP “London” mutant and develop abundant amyloid plaques later in life (15). The discrepancy between these studies could be explained by a cross-species variability of APP and Aβ. Namely, it has been found that in mice and rats, APP is less processed by the β-secretase pathway than in primates. The Aβ peptides in rodents differ in three amino acids from Aβ peptides in humans and display lower tendency to aggregate. In contrast, the APP molecule in guinea pig shares high degree of homology with human APP, its processing is very similar to that of human APP, and the Aβ sequence is identical to that of human Aβ (14). Additional in vivo evidence is necessary to clarify the relationship between secretory APP processing induced by PKC activation and Aβ generation.

The presenilin (PS) 1 and PS2 proteins are transmembrane proteins that are critically involved in γ-
secretase cleavage of APP (16). Mutations in the PS genes that cause familial forms of early onset Alzheimer’s disease lead to increased production of Aβ42. Very recent data has identified a phosphorylation site for PKC within a recognition motif for caspases in PS1 (9). Phosphorylation at this site by PKC regulates the caspase-mediated cleavage of PS1 and inhibits the progression of apoptosis. These new findings support an anti-apoptotic role of PKC. Furthermore, different PKC isoforms seem to be involved in cell survival. Studies show that PKC(α) is able to phosphorylate Bcl-2, an anti-apoptotic protein, at a site that increases its anti-apoptotic function and overexpression of PKC(α) results in increased expression of Bcl-2 (8).

**Aβ accumulation and PKC signaling**

Intraneuronal accumulation of Aβ occurs in normal aging without deposition of Aβ in amyloid plaques (3) and/or associated with extracellular deposits in the AD. The mechanisms underlying this process might be primarily related to a deficiency of an intracellular signal transduction system that develops with normal aging and is amplified by the AD risk factors. Immunohistochemical studies in aged non-demented subjects have shown that neurons with marked intracellular Aβ42 immunoreactivity also stain positive for apolipoprotein E (ApoE) (4), a major risk factor for late-onset of AD (17). A key signal transduction system that generally declines with aging is PKC. Consistent alterations of PKC level (18), PKC activity (19), and anchoring mechanisms for PKCs to subcellular compartments through receptors for activated C kinase (RACK1) (20) have also been reported in the postmortem tissue from AD brains. Not all aged individuals develop AD, but aging seems to be a prerequisite of AD. It can be hypothesized that in aging in the presence of high risk AD factors, PKC deficiency would unbalance the APP-α-processing towards a β- and/or γ-processing with generation of soluble Aβ. Therefore, it is reasonable to think that gradual elevation of soluble Aβ will initially activate PKC and related down-stream pathways, while high constant level of Aβ, as in the late stage of AD, will downregulate PKC and dampens the PKC-related intracellular pathways. Clinical and experimental studies show hyperactivation of PKC in AD patients and in animal models. Increased immunoreactivity for PKC(βII) has been found in diffuse and senile amyloid plaques (6). A recent study in postmortem AD brain tissue has found increased mitogen-activated kinases (MAPK) activity, an intracellular enzyme located downstream to PKC that could be responsible for the enhanced phosphorylation of tau protein and subsequent generation of neurofibrillary tangles (NFT) (21). Transgenic mice that overexpress the Swedish mutant of human APP695, Tg2576, develop numerous cortical and hippocampal Aβ plaques at 9–13 months of age and show an activation of multiple isoforms of PKC involved in APP processing (PKCα) and neuronal survival (PKCγ, PKCζ) (22). In contrast, by 20 months of age, Tg2576 mice show decreased extracellular signal-regulated kinase (ERK2) MAPK and reduced activation of transcription factor cAMP-response element binding protein (CREB) (23). We have recently shown that chronic exogenous administration of soluble Aβ into the rat cerebral ventricle increases radioreceptor binding to phorbol dibutyrate (PDBu), a measure of enhanced PKC activation, and also decreases the activity and translocation of PKC to the cellular membrane in the hippocampus (24). The dual effect of Aβ on PKC in our experiment could be explained by a chronic delivering system (for review, see ref. 25). In the first days of infusion, low accumulation of Aβ could activate PKC enzyme but a continuous delivery system would increase the Aβ level that could stimulate permanently PKC and induce its down-regulation. The enzyme would be long-lasting bound to membrane and thus unable to respond to further stimulation. Decreased activity of PKC in our non-transgenic animal model is not secondary to an Aβ-induced neurodegeneration because studies on this model do not find cell death in the hippocampus (for review, see ref. 26). Rather we suggest that PKC alteration is a primary event in AD progression because other studies on peripheral tissue from AD patients have shown reduced PKC activity in non-neuronal cells such as fibroblasts (10). Ultimately, the decline in the intracellular signal transduction systems in AD that lead to decreased activation of transcription factors and protein synthesis may cause cell death and memory loss.

**Cell death, neurogenesis, and Alzheimer’s disease**

Memory loss associated with late stages of AD is believed to be caused by neuronal degeneration in cognition-related brain regions. In the AD brain, large-scale cell death of mature neurons is a pathologic process that remains unsolved. Recently, new types of cell death have been proposed for neuronal loss specifically through apoptosis and cell cycle reentry. Aβ protein could be a driving force in these processes. The amyloid protein induces apoptosis through oxidative stress while also driving cell division and cell death in cultured neurons (27). Evidence for DNA fragmentation, expression of apoptosis-related genes, and caspase activation support an apoptotic mechanism in AD neurodegenera-
tion. Studies have shown that several atypical isoforms of PKC might suppress apoptosis induced by Aβ and promote survival (8). Potential therapeutic agents for AD based on PKC activators have been also proposed in clinical studies (15). Very recent lines of evidence have suggested that mature neurons degenerate in AD and in mild cognitive impairment (MCI) because they reenter a cell cycle they are unable to complete (28). Immunohistochemical data from AD tissue has revealed reexpression of cell cycle related-proteins in neurons undergoing cell death. There is also direct evidence that neurons in AD enter the cell cycle but do not complete mitosis and remain tetraploid. These poplyploid neurons survive for several months after their genome is replicated but ultimately die through an apoptotic process. To date there is no direct link between cell cycle activation and PKC, but cell culture studies have shown that PKC along with other kinases is required for vascular endothelial growth factor (VEGF)-induced proliferation of neuronal precursors (29).

Although AD is characterized by neuronal degeneration and cell loss, recent studies have revealed increased neurogenesis in the dentate gyrus and CA1 area in AD brains (30). Increased generation of new neurons in the hippocampus could be a secondary event to the massive cell loss observed with the progression of the disease. Normal aged brain has markedly reduced hippocampal neurogenesis but interestingly, preserves the potential to increase the production of new neurons in certain conditions (31). Alternatively, neurogenesis might be directly stimulated by Aβ in AD brains. Determining which mechanism underlies the enhanced neurogenesis in AD brain needs further elucidation, but these findings leave open the question whether the stem cell-based therapies in AD could be a practical therapeutic issue to focus on.

Conclusions

Aβ has a central role in the pathogenesis of AD. Generation of non-amyloidogenic fragments of the amyloid protein is controlled by PKC, an enzyme that is downregulated during aging. In elderly individuals, decreased PKC activity in concert with increased AD risk factors could set up the stage for accumulation of Aβ in neurons and progression of AD. Consequently, decreased intracellular signaling pathways controlled by PKC could reduce neuronal survival, increase cell death, and induce memory loss in AD. Drugs developed as PKC activators are of potential therapeutic interest for AD but additional studies are needed to support this idea. Recent findings that the AD brain is able to generate more new neurons than the normal adult brain bring also a new and exciting view into stem cell therapy. The mechanisms that control this process must still be identified and more importantly, whether it can be translated into a practical approach for the AD therapy.

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References

13 Rossner S, Mendel K, Schliebs R, Bigl V. Protein kinase Cα and β1 isoforms are regulators of α-secretory proteolytic processing


