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PKC signaling deficits: a mechanistic hypothesis for the origins of Alzheimer's disease

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There is strong evidence that protein kinase C (PKC) isozyme signaling pathways are causally involved in associative memory storage. Other observations have indicated that PKC signaling pathways regulate important molecular events in the neurodegenerative pathophysiology of Alzheimer's disease (AD), which is a progressive dementia that is characterized by loss of recent memory. This parallel involvement of PKC signaling in both memory and neurodegeneration indicates a common basis for the origins of both the symptoms and the pathology of AD. Here, we discuss this conceptual framework as a basis for an autopsy-validated peripheral biomarker – and for AD drug design targeting drugs (bryostatin and bryologs) that activate PKC isozymes – that has already demonstrated significant promise for treating both AD neurodegeneration and its symptomatic memory loss.

Introduction

Alzheimer's disease (AD) is clinically unique in that it is the only dementia that commonly begins with a 'pure' loss of recent memory in the absence of any other cognitive deficit. Unlike other dementias, AD often begins as a disease of memory. Other dementias such as those resulting from vitamin B-12 deficiency, Parkinson's disease, Huntington's chorea or multiple strokes are associated with other symptoms such as paresthesias and movement disorders.

AD, as a disease of memory, is also uniquely associated with two pathological hallmarks in the brain: extracellular amyloid plaques and intracellular neurofibrillary tangles. Amyloid plaques have been shown to arise from aggregation of the soluble protein known as β-amyloid ($A\beta_{1-42}$) and other variants such as $A\beta_{1-40}$. Amyloid plaques arise after soluble $A\beta_{1-42}$ monomers begin to oligomerize into progressively larger aggregations [1] that then form fibrils followed by plaques. The amyloidogenic Aβ fragment results from β-secretase-mediated cleavage of amyloid precursor protein (APP) to generate an NH_2 terminus [2] and a further cleavage by γ-secretase to generate the Aβ peptide, which has a length of 40, 42 or 43 amino acids [3]. Cleavage of APP by α-secretase at a different site generates soluble APP (sAPP), which is generally considered to be nontoxic. Most evidence suggests [4] that sAPP production by α-secretase competitively reduces Aβ production by the β- and γ-secretases. Protein kinase C (PKC) isozymes α and ε and possibly other isozymes (Box 1, Table 1) [5–10] can activate the α-secretase-mediated cleavage of APP directly, or indirectly through phosphorylation of extracellular-signal-regulated kinase (ERK1/2).

Accumulation of another protein, hyperphosphorylated tau, in AD pathology occurs in neurofibrillary tangles (NFTs) within cell bodies and 'threads' in axons. These neurofibrillary tangles can lead to neuronal death and subsequent release of the tangles into the extracellular environment. NFT accumulation seems to be induced by hyperphosphorylation of tau and its subsequent disengagement from microtubules. The enzymes directly involved in tau hyperphosphorylation are ERK1/2 and glycogen synthase kinase 3β (GSK-3β), both regulated by PKC. Therefore, PKC isozymes α and ε directly or indirectly regulate all of the major enzyme pathways that are responsible for post-translational processing of APP (Figure 1) involving cleavage by α, β or γ secretase (or combinations thereof) in addition to those enzyme pathways responsible for processing tau.

The causal role of PKC in AD was first suggested when PKC isozymes were found to be deficient in postmortem brain samples from AD patients. Other evidence involved abnormalities of PKC isozyme function in human skin fibroblasts of AD versus control patients [10–13] in addition to abnormalities of PKC-regulated K^+ channel function [14] and PKC-activated phosphorylation of the mitogen-activated protein kinases (MAPKs) ERK1/2 [15]. In a recent study of human skin fibroblasts, the natural PKC activator, bradykinin, induced abnormalities of ERK1/2 phosphorylation that were diagnostic of AD. Furthermore, autopsy validation confirmed this abnormality of PKC-mediated phosphorylation of ERK1/2 as most marked in the earliest stages of AD [16]. These results suggest that PKC-mediated abnormalities of ERK1/2 phosphorylation are induced in the initial stages of AD, perhaps as part of an early inflammatory process.

Because of the crucial role of PKC in the amyloid and tau processing pathways, in addition to its direct participation in associative memory storage, a systemic deficiency in the

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PKC–mitogen-activated protein kinase (MAPK)–ERK1/2 pathway could explain the symptomatic memory loss of AD and many other aspects of AD pathology. A deficiency of PKC activation could then account for:

(i) Memory loss – the characteristic presenting symptom of AD.
(ii) Increased levels of Aβ (owing to decreases in the activity of PKC, MAPK and α-secretase) and resulting amyloid plaques.
(iii) Increased levels of phosphorylated tau (owing to reduced PKC-mediated inhibition of GSK-3β) and resulting neurofibrillary tangles.
(iv) Inflammation – AD might particularly involve early inflammation signaling triggered by natural mediators such as bradykinin that activate PKC and, in turn, tumor necrosis factor α (TNF-α) and interleukins, and phosphorylation of ERK1/2.

Evidence to support a primary mechanistic role of PKC in AD, as discussed later, includes the therapeutic efficacy of drugs that activate PKCα, ε and δ, the early diagnostic accuracy for AD of PKC-mediated ERK1/2 phosphorylation, and a human cell model of AD induced by $A\beta_{1-42}$, which is reversed by the PKC activator bryostatin.

PKC signaling deficits: a locus for AD-specific memory loss

Implication of the crucial role of PKC signaling in associative memory storage for several animal models [17,18] led to the hypothesis that deficits of PKC could be a molecular locus for the short-term memory loss of AD. PKC was first causally implicated in signaling pathways in Pavlovian conditioning of the nudibranch *Hermissenda* [19], and then in rabbit nictitating membrane conditioning [20], rat spatial maze learning and olfactory discrimination learning, and cortex-dependent visual discrimination learning [21]. These mammalian associative memory models produce learning-specific changes of endogenous PKC levels within those brain structures that lesion studies had previously identified as required for memory storage (Figure 2) [18,20,21].

Electrophysiologic measures demonstrated memory-specific changes of membrane currents and synaptic facilitation that could be induced by application of exogenous PKC activators [17,22] and were consistent with PKC-mediated enhancement of synaptic potentials in hippocampal and cerebellar dendrites and dendritic branches [17,24]. Other changes of endogenous PKC signaling molecules during these memory paradigms included upstream PKC regulators such as insulin, the insulin receptor [25] and fibroblast growth factor 18 [18,26], and downstream PKC substrates such as the MAPKs ERK1/2, the type II ryanodine receptor, Src non-receptor tyrosine kinase [25,27], the mRNA stabilizing proteins human antigen D (HuD), HuC and HuB [28], and proteins whose mRNA is protected by HuD proteins, such as growth-associated protein 43 (GAP43). Other studies

Box 1. Protein kinase C

Protein kinase C isozymes

Protein kinase C (PKC) comprises a multigene family of phospholipid-dependent, serine/threonine protein kinases. In mammals, 12 PKC isozymes (Figure Ia) have currently been identified, with various PKC isozymes being usually coexpressed in the same neurons. Based on their molecular structures and sensitivity to activators, PKC isozymes are divided into three subfamilies: classical PKC (cPKC: α, β_I, β_{II} and γ); novel PKC (nPKC: δ, ε, η and θ); and atypical PKC (aPKC: ζ and λ/ι). The cPKC isozymes require both Ca^{2+} and phosphatidylserine, diacylglycerol or other activators for activation. They contain four homologous domains: two regulatory (the activator-binding C1 domain and the cofactor Ca^{2+}-binding C2 domain) and two catalytic (the C3 domain, containing the ATP-binding site, and the C4 domain, containing the substrate-binding site), interspaced with the isozyme-unique (variable, or V) regions. The nPKC isozymes lack the C2 domain and are Ca^{2+}-independent in activation. The cPKC and nPKC isozymes can thus be activated by diacylglycerol, phorbol esters and bryostatins, with cPKCs requiring Ca^{2+} as the cofactor for activation. The aPKC isozymes lack both the C2 domain and half of the C1 homologous domains and are insensitive to Ca^{2+}, diacylglycerol, phorbol esters or other PKC activators. All the PKC isozymes contain, near the C1 domain, an N-terminal pseudosubstrate motif, which binds to the catalytic domain in the inactive state, and functions as an autoinhibitory domain of PKCs. Each of the PKC isozymes is encoded by a separate gene, with the exception of the β_I and β_{II} isozymes, which are alternative splice variants.

Activation and degradation of protein kinase C

Activation of PKC depends on its catalytic competence and its targeting to membrane compartments (Figure Ib). Catalytic competence is achieved by three sequential phosphorylation steps involving the activation loop, the turn motif, and the hydrophobic motif [5]. Targeting to membrane compartments is promoted by second messengers such as diacylglycerol and arachidonic acid and is stabilized by specific anchoring proteins (e.g. RACK1) [6]. For PKC isozymes α and ε, proteolytic degradation begins when dephosphorylation occurs at the three 'priming' sites required for activation. Phosphorylated PKC enters the ubiquitin-proteasome degradation cascade [7]. Furthermore, there is evidence that another degradation pathway involves caveolae-dependent trafficking of the active enzyme and subsequent proteasome-independent degradation. Bryostatin, a potent PKC activator, triggers both proteasome-dependent and proteasome-independent pathways for PKCα and ε degradation following PKC activation. Degradation (i.e. 'downregulation') of PKC isozymes is then followed by a prolonged increase of PKC isozyme synthesis. Bryostatin and phorbol esters bind to the same site on PKC, the diacylglycerol (DAG)-binding site. However, the DAG-binding site is complex, and marked differences are observed among the PKC isozymes. Lewin *et al.* [8] showed that bryostatin and phorbol 12,13-dibutyrate (PDBu) differ markedly in their binding to PKC. The binding affinity of bryostatin was too high to measure and the rate of release was much slower than phorbol ester ($t_{1/2}$ = several hours). This caused bryostatin to inhibit [^{3}H]PDBu binding in a noncompetitive manner. The greater affinity of bryostatin for PKC causes sustained activation of PKC. PKCα in particular might be resistant to downregulation by bryostatin [9]. Bryostatin is more effective than phorbol esters in downregulating PKC.

Both conventional and novel isoforms of PKC are activated by the binding of the natural PKC activator diacylglycerol to the cysteine-rich C1A and C1B domains of PKC. Bryostatin or phorbol esters also produce activation by binding to the same activation site. In the presence of calcium, activation results in rapid translocation to the membrane fraction, where PKC undergoes autophosphorylation. Endogenous phosphatases eventually dephosphorylate the PKC, initiating the downregulation phase, which might occur by ubiquitination and degradation by the proteasome, or by translocation of PKC to caveolae and delivery to endosomes, where it undergoes proteolytic degradation.

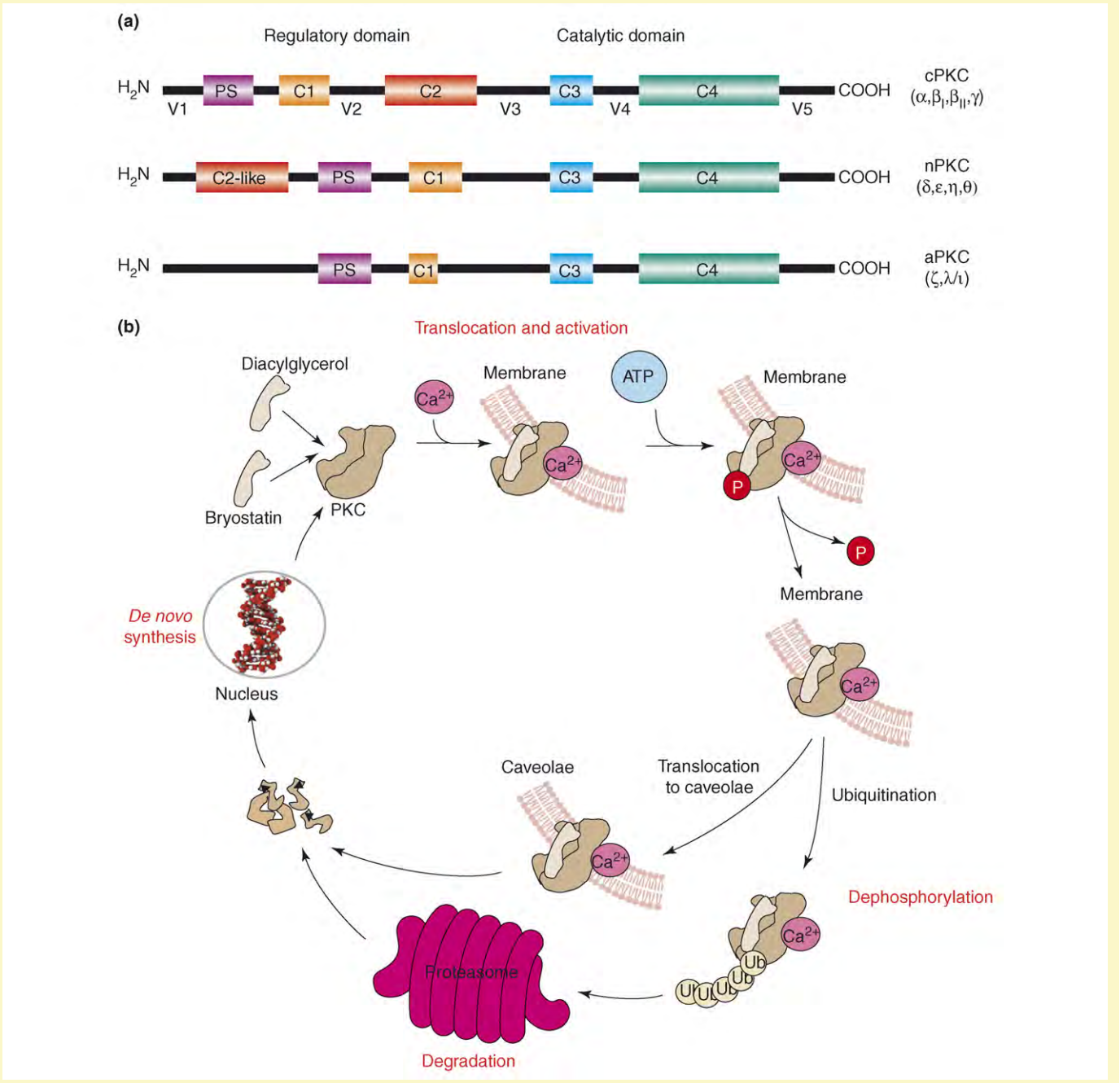

Figure I. Protein kinase C: activation–downregulation cycle.

include genetic mutations of PKC isozymes in *Drosophila*, endogenous PKC activation specific to memory of bees [29], transgenic mice with PKC mutations, and PKC-mediated long-term potentiation (a synaptic model of memory) [30]. Disruption of PKC function can also interfere with associative learning and memory. Vianna *et al.* [31], for example, showed that brain infusion of various PKC inhibitors prevented retrieval of previously stored memories and blocked both long-term and short-term memory in an isoform-specific manner.

More recently, PKC activation by the potent PKC activator bryostatin was shown in *Hermissenda* to induce the synthesis of those proteins required for long-term memory consolidation, in advance of the training events themselves [32]. When PKC was activated before training by administering bryostatin, long-term memories could be formed even in the presence of protein synthesis inhibitors. PKC regulation of protein synthesis required for memory consolidation can be effected through different pathways such as those involving the MAPKs ERK1/2, nuclear factor κB (NF-κB), Wnt and the PKC substrates HuD, HuC and HuR.

Table 1. PKC signaling deficits

PKC signaling deficits initially cause	Potential consequences in Alzheimer's disease
Dysfunctional PKC–MEK–ERK1/2	Recent memory loss
Aβ elevation; Aβ oligomers	Amyloid plaques
Hyperphosphorylated tau protein	Neurofibrillary tangles
TNF-α, interleukins	Inflammation

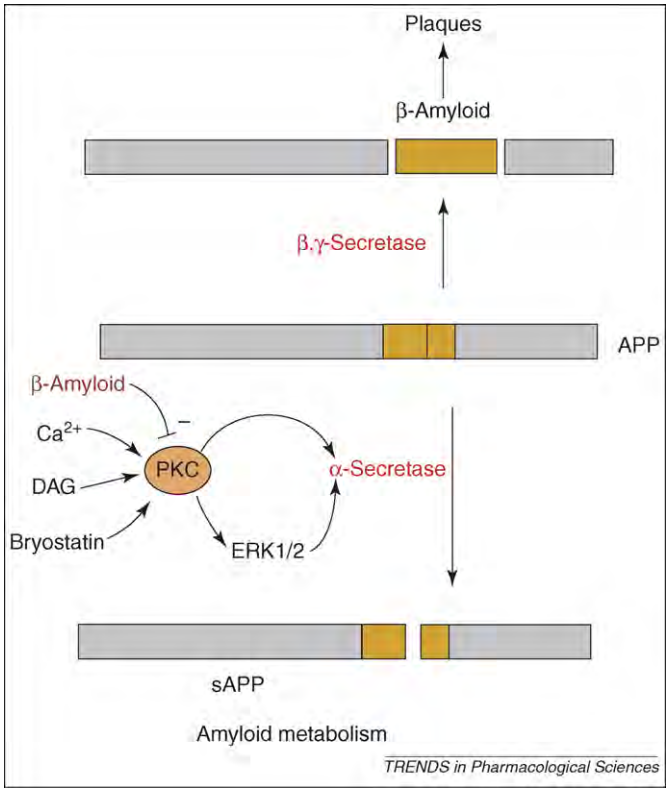

Figure 1. Amyloid metabolism. Amyloid precursor protein (APP) can be digested by β- and γ-secretases in the amyloidogenic pathway, which releases the toxic β-amyloid peptide, or in the α-secretase pathway, which releases sAPP. Because α-secretase cleaves near the middle of the β-amyloid sequence, formation of sAPP and β-amyloid are mutually exclusive. Protein kinase C (PKC) activates α-secretase indirectly through the MAP kinases ERK1/2, and can also activate α-secretase directly. Bryostatin, diacylglycerol (DAG) and calcium ions activate PKC, whereas β-amyloid directly inhibits PKC and causes downregulation of PKC activity by a pathway involving the proteasome and possibly also requiring the synthesis of additional unknown factors.

PKC also phosphorylates molecular substrates that are directly involved in regulation of synaptic connections (Figure 3) [33,34], which are the physiological loci of memory storage. Thus, PKC dysfunction in AD could directly alter synaptic function within presynaptic and postsynaptic compartments. Loss of synapses and synaptic function in AD, particularly of acetylcholine-containing synapses [35], is partly an inevitable consequence of the cell loss and the loss of Ca^{2+} homeostasis in AD. However, some evidence suggests that synapses themselves, which are highly dependent on intact cytoskeletal structure, could be particularly vulnerable in AD. For example, abnormalities in the postsynaptic dendritic spines have been observed in APP transgenic mice (a model for AD). Because of the devastating effects of AD on memory, it is not surprising that dendritic spines are also a significant site of AD pathology [36].

The role of PKC in amyloid processing

As introduced above, the major means of activating α-secretase-mediated cleavage of APP is either direct by activation of PKC isozymes α and ε, or indirect through PKC activation of ERK1/2, or both (Figure 4a) [4,37,38]. Generation of sAPP by α-secretase cleavage of APP occurs at the outer cell membrane, whereas Aβ generation by β- and γ-secretase-mediated cleavage of APP occurs to a significant degree in the trans-Golgi network. PKC activation (by phorbol 12-myristate 13-acetate, or PMA) reduces Aβ secretion and increases sAPPα secretion through α-secretase activation [39]. PMA-induced α-secretase activation apparently involves translocation of the α-isozyme of PKC from the cytosolic to the membrane compartment, and translocation of the ε-isozyme of PKC from cytosolic to Golgi-like structures.

Although PKC can work directly on α-secretase molecules such as the ADAM (a Disintegrin and Metalloproteinase) family member tumor necrosis factor-α converting enzyme (TACE) or other α-secretases such as ADAM-10 [40], the MAPKs ERK1/2 (which are also phosphorylated by PKC) have been shown to phosphorylate TACE directly within a specific molecular domain. Because most secreted Aβ is likely to be produced in the trans-Golgi network (TGN), whereas intracellular $Aβ_{1–42}$ is produced in the endoplasmic reticulum (ER) intermediate compartment (IC), both β- and α-secretases are apparently active in both ER-IC and TGN [41] compartments, and at the plasma membrane.

To date, it has not been possible to identify whether TACE or ADAM-9 or -10 are the major α-secretases functioning *in vivo* and activated by PKC [41–43]. The phosphorylation involved in α-secretase cleavage of APP at the cell surface (i.e. at the plasma membrane) is greatly enhanced by either PKC or ERK1/2 stimulation. This cell surface cleavage of APP leads to 'shedding' of the APP ectodomain [44]. Although the MAPKs have direct phosphorylation sites on α-secretase, their effect on APP processing remains to be clarified. Even less is known about the phosphorylation effect of PKC on α-secretase, because sites of direct phosphorylation by PKC as yet remain unidentified. Although protein phosphorylation is required for α-secretase activation [45] and this phosphorylation involves PKC and ERK1/2 pathways, statin (cholesterol-lowering drugs)-induced increases of sAPP release (through α-secretase) are not blocked by either inhibitors of PKC or ERK1/2 [46], indicating that α-secretase can also be activated by a mechanism that is independent of PKC and MAPKs.

The role of PKC in tau processing and neurofibrillary tangles

As introduced above, tau, a structural protein that binds to and stabilizes tubulin in axonal microtubules, becomes hyperphosphorylated and forms insoluble paired helical filaments that accumulate inside neuronal cell bodies. These accumulations, called neurofibrillary tangles, together with amyloid plaques, are associated with progressive loss of neurons and synapses and dementia [47,48].

Fibrillar $Aβ_{1–42}$ induces ERK1/2 activation, which in turn can lead to hyperphosphorylation of tau and subsequent neurodegeneration. Furthermore, the toxic oligomeric forms of Aβ that can cause synaptic dysfunction induce neuronal death through activation of the ERK1/2 pathway and subsequent proteolytic cleavage of tau. Although ERK1/2 can cause tau phosphorylation, the principal kinase that phosphorylates tau is GSK-3β [49]. PKC can inhibit GSK-3β directly, thus reducing

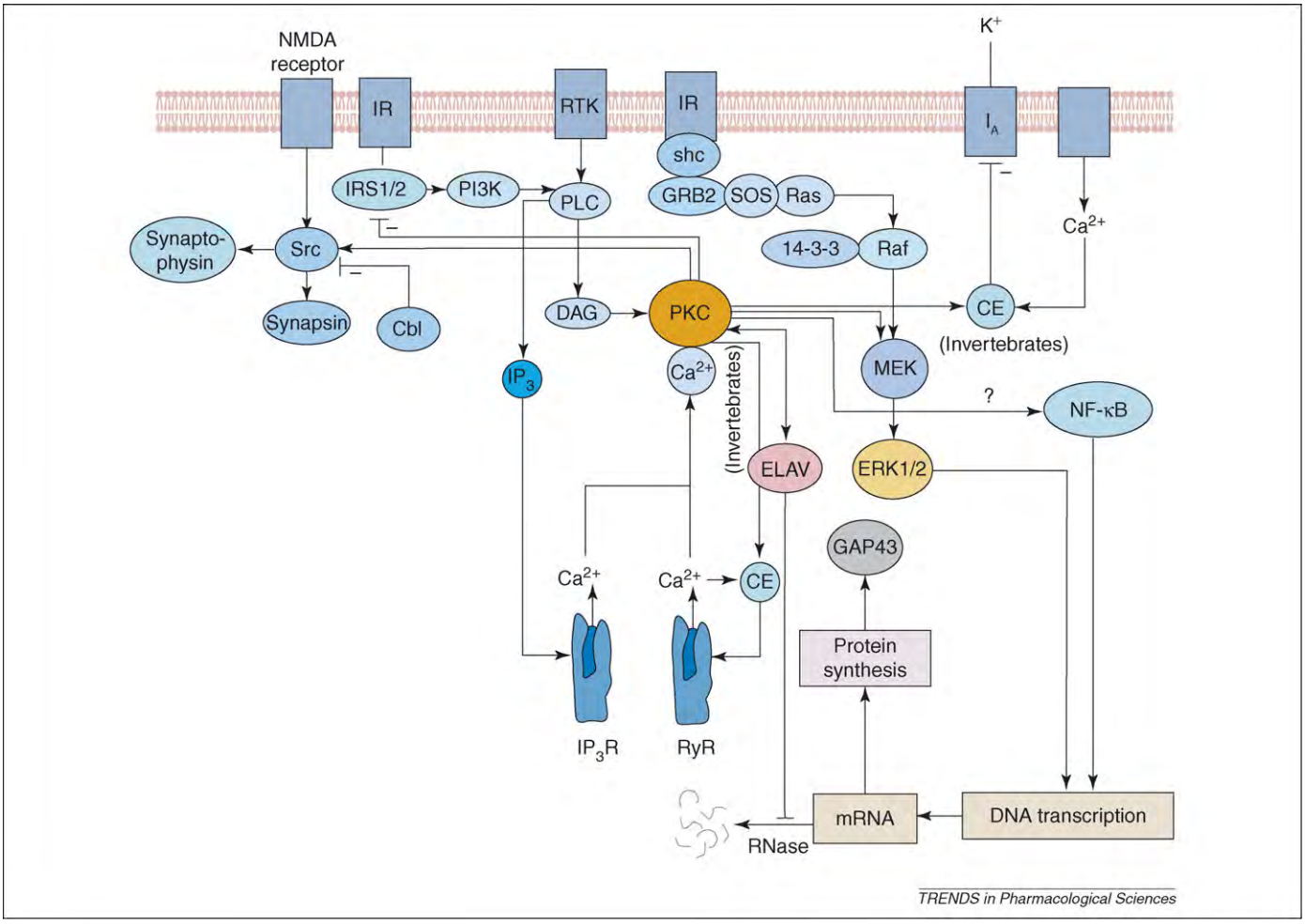

Figure 2. PKC pathways in memory. During learning, PKC is activated and translocates to the membrane fraction. PKC activates the MAP kinases ERK1/2 and NF-κB, leading to increased DNA transcription. The role of PKC in associative memory was first suggested by detection of calcium-dependent phosphorylation of protein substrates in the eye of the sea slug *Hermissenda* after Pavlovian conditioning. PKC-dependent phosphorylation of a 22-kDa PKC substrate, calexcitin (CE), causes inactivation of voltage-dependent K^+ currents, I_A and I_{Krect}, and produces conditioning-specific changes of membrane excitability [22] and synaptic facilitation in postsynaptic interneurons [23]. The closest homolog to CE in mammals is calsenilin, also a PKC substrate, which binds to presenilin, and which is part of an APP-cleaving secretase implicated in AD. In neurons, influx of calcium through Ca^{2+} channels, produced by either calexcitin- or calsenilin-mediated inhibition of K^+ channels, activates calcium-induced calcium release from the endoplasmic reticulum by activating the ryanodine receptor (RyR; also activated by calexcitin or calsenilin) and the inositol-1,4,5-trisphosphate (IP_3) receptor. PKC and calcium can activate DNA transcription directly or indirectly through NFAT (nuclear factor of activated T cells), NF-κB, CREB (cAMP-response element-binding protein), and the Fos-Jun transcription factors. PKC interacts with the ELAV (embryonic lethal abnormal visual system) mRNA-binding proteins, including HuB, HuD and HuC. These RNA-binding proteins stabilize mRNA, leading to increased synthesis of learning-related proteins such as GAP43 and PKC. The insulin receptor (IR) is upregulated in CA1 pyramidal cells during learning and the insulin receptor substrate (IRS) could have a modulatory role in memory by activating the Src non-receptor tyrosine kinases. Src also interacts with tau (not shown). The insulin receptor substrate and insulin-like growth factors also activate the phosphatidylinositol 3-kinase (PI3K)–Akt signaling pathway. Activating the Akt signaling pathway produces, among other things, an activation of NF-κB, inhibition of GSK-3β and an increase in levels of cADPR (cADP-ribose), which activates the ryanodine receptor. Abbreviations: GRB2, growth factor receptor-bound protein; PLC, phospholipase C; RTK, receptor tyrosine kinase; SOS, son of sevenless.

tau phosphorylation and neurofibrillary tangles [50], and indirectly through its effects on Aβ (Figure 4a). PKC indirectly inhibits GSK-3β by reducing production of $Aβ_{(1–42)}$, which is an activator of GSK-3β (Figure 4b). The reduction in levels of $Aβ_{1–42}$ by PKC through an α-secretase-mediated increase of sAPP, the inhibition by PKC of GSK-3β and thus tau phosphorylation, together with Aβ-mediated activation of GSK-3β, collectively provide a direct mechanistic connection between amyloid plaques and neurofibrillary tangles.

PKC-mediated inflammation in neurodegeneration

Although inflammation is often viewed as a secondary consequence of cell injury, new evidence indicates close ties between inflammation, PKC and β-amyloid production that could be important in early AD. Recently, bradykinin, an inflammatory signal in the brain and peripheral tissues, was shown to elicit AD-specific, PKC-mediated phosphorylation of ERK1/2 [16]. Furthermore, AD apparently involves cognitive-impairment-correlated alterations of the plasma levels of inflammation factors such as TNF-α or interleukin 1β (IL-1β), both of which interact with PKC [51]. These results suggest that dysfunction of PKC-mediated activation of α-secretase contributes to abnormalities of inflammatory signaling in early AD pathophysiology. PKC signaling is also a target for oxidative stress and derangements of cholesterol metabolism. The cysteine-rich C1B domain of PKC behaves in some respects like a redox sensor, causing PKC to be activated by hydrogen peroxide in a manner independent of diacylglycerol [52]. The oxysterol 7β-hydroxycholesterol is a potent inhibitor of TACE, which is not only an important

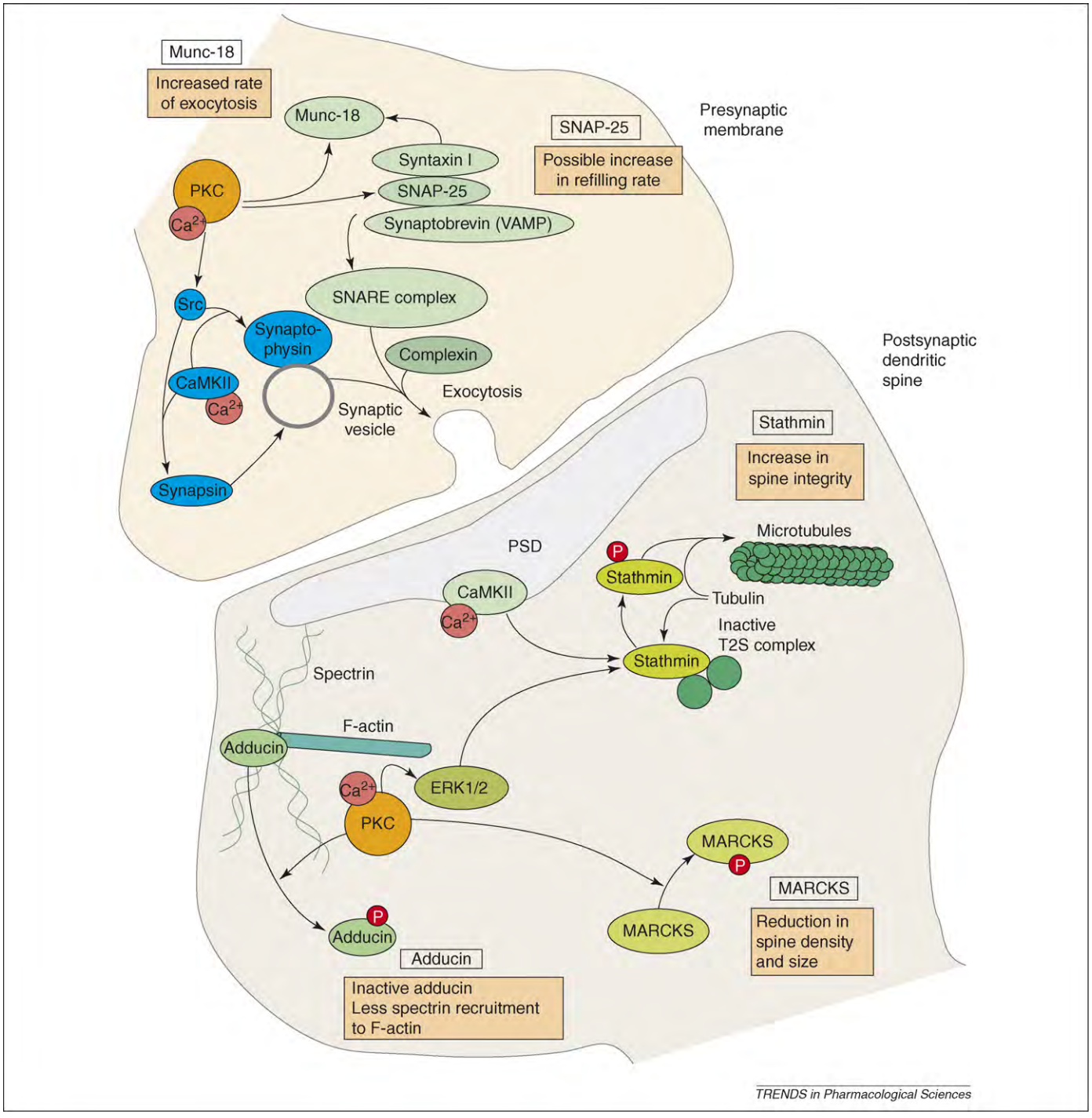

Figure 3. Effects of PKC-mediated protein phosphorylation in presynaptic and postsynaptic compartments. Presynaptic and postsynaptic compartments have been implicated by many studies as loci for memory-induced changes. In the postsynaptic dendritic spine, phosphorylation by PKC and Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII) affect spine integrity by acting on adducin, stathmin and MARCKS (myristoylated alanine-rich C-kinase substrate). Phosphorylation of adducin [33] reduces the affinity of adducin for spectrin, and reduces the ability of adducin to recruit spectrin to the ends of actin filaments. Phosphorylated adducin also moves from the postsynaptic density (PSD) and cell membrane to the cytosol, where it is less effective at promoting actin capping. Phosphorylation of MARCKS also produces a reduction in spine density and size. Phosphorylation of stathmin (also known as oncoprotein 18), by contrast, has an activating effect. Dephosphorylated stathmin binds and sequesters two molecules of tubulin to form an inactive T2S complex, which inhibits tubulin polymerization. After phosphorylation by CaMKII or the MAP kinases ERK1/2, stathmin dissociates from tubulin, enabling microtubules to form. Stathmin interacts with several other proteins besides tubulin. After associative learning, the amount of stathmin undergoing protein interactions increases over fourfold. PKC-induced liberation of tubulin could produce an increase in spine integrity, stabilizing some types of dendritic spines. Other types of spines could be destabilized by PKC and other kinases. The pathways shown here are only a subset of the biochemical steps important for the structural integrity of synaptic spines. In the presynaptic membrane, the role of phosphorylation on vesicle release is less clear; although Ca^{2+} is essential for exocytosis, phosphorylation by PKC, CaMKII and A-kinase could have an important modulatory role. Exocytosis requires the SNAP receptor SNARE (soluble *N*-ethylmaleimide-sensitive fusion protein attachment protein receptor) complex, which comprises 25-kDa synaptosomal-associated protein (SNAP-25), syntaxin 1 and synaptobrevin (VAMP, or vesicle-associated membrane protein). Synaptobrevin, a possible PKC substrate, acts as a calcium sensor for exocytosis. Phosphorylation of SNAP-25 by PKC has several effects on the affinity of SNAP-25 for syntaxin *in vitro*, but the role of SNAP-25 phosphorylation in vesicle release is still not well understood. PKC is known to phosphorylate Munc18 (mammalian homolog of uncoordinated protein 18) on Ser313, which reduces the affinity of Munc18 for syntaxin 1A. PKC might also phosphorylate 14-3-3 proteins and a variety of actin-binding proteins (not shown) to produce rearrangements of the actin cytoskeleton. Complexin is also essential for calcium-dependent synaptic vesicle exocytosis. After associative learning, complexin experiences a decrease in protein–protein interactions with two other proteins, which could free complexin to facilitate vesicle release. PKC, along with other kinases such as CaMKII, also indirectly increases the phosphorylation of synaptophysin, a protein associated with synaptic vesicles,

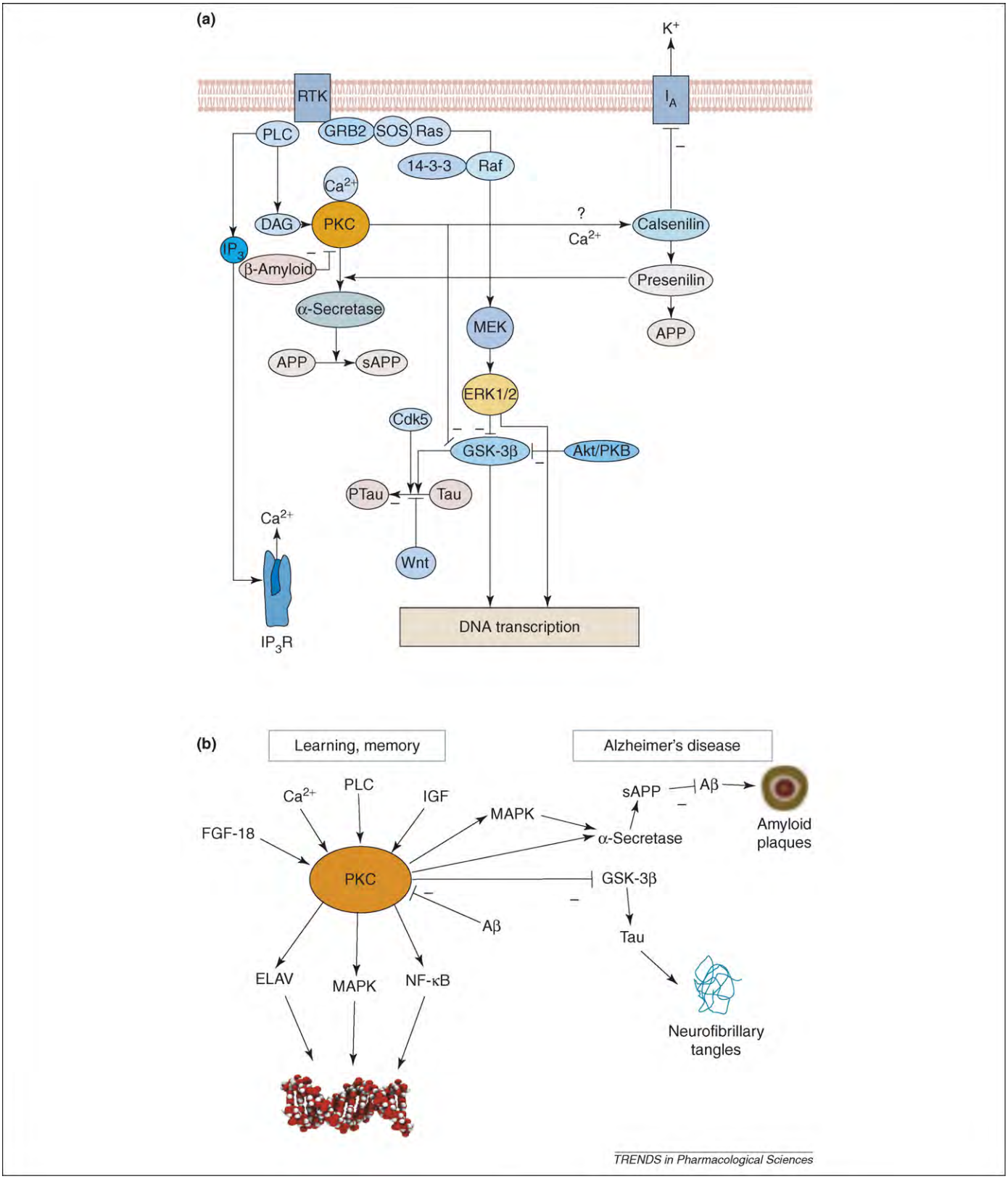

Figure 4. Overview of AD and learning pathways. **(a)** Biochemical pathways associated with Alzheimer's disease (AD). Many of the pathways involved in learning could also be impaired in AD. In AD, protein kinase C (PKC) activates α-secretase, which causes proteolysis of amyloid precursor protein (APP) to create sAPP, while simultaneously

and synapsin, a protein that is normally associated with cytoskeletal actin. After phosphorylation of synapsin, synaptic vesicles dissociate from the filamentous network and are able to participate in exocytosis. Memory-specific protein–protein interactions [34] involved in synaptic regulation or remodeling (or both) are also implicated in PKC signaling pathways, such as increased binding of Src non-receptor tyrosine kinase to synapsin, synaptophysin and the NMDA receptor, and spectrin interactions that are regulated by PKC involving the PKC substrate adducin. Many of these protein–protein interactions have also been implicated in PKC-mediated regulation of postsynaptic dendritic spines or presynaptic terminal endings, or both.

α-secretase, but also the rate-limiting enzyme in the synthesis of the inflammatory signal TNF-α [53].

Concluding remarks

Many recent findings support PKC signaling deficits as a common mechanistic basis for all of the major elements of AD (Table 1).

PKC-mediated ERK1/2 phosphorylation has recently been shown (see above), with autopsy confirmation, to be specific for AD compared with age-matched controls and other non-AD dementias in skin fibroblasts. Furthermore, Aβ, the toxic metabolic degradation product of cleavage of APP by β- and δ- secretases, inactivates PKC [13]. Recently, this inactivation was shown to arise from direct binding of Aβ to PKC at positions 28–32 of the amino acid sequence of Aβ [54]. In AD patients who are already prone to elevation of Aβ concentration, Aβ feedback inhibition on PKC would be expected to cause still greater reduction of α-secretase activity and thus further production of Aβ. Abnormal Aβ-mediated inhibition of PKC would be expected to impair memory storage and promote AD neurodegeneration directly. Protein kinase Mζ, formerly considered to be an atypical isoform of PKC, was found to localize in limbic neurofibrillary tangles in AD patients. Overexpression of PKCε reduced levels of Aβ in AD transgenic mice [55]. Finally, age, the single greatest risk factor for the sporadic form of AD (accounting for 90–95% of cases), has been associated with progressive compromise of PKC function. Aged animal models, for example, have shown age-specific changes of PKC isozyme distribution in the brain, impaired PKC translocation, reduced levels of the PKC anchoring protein, RACK1 (receptor for activated C kinase 1) [56], alterations in ERK1/2, and reduced levels of the α-secretase-cleaved APP product, soluble APPα, in the cerebrospinal fluid [57].

These conclusions all point to the potential efficacy of PKC activators for treating the symptoms and neurodegeneration of AD. Bryostatin, a nontumorigenic and potent activator of PKC isozymes, has been shown to enhance memory and reduce brain levels of Aβ. Bryostatin greatly facilitates and prolongs Pavlovian conditioning of the sea slug *Hermissenda*, Pavlovian conditioning of the rabbit nictitating membrane, and rat spatial maze learning [58]. In addition, bryostatin has also been shown to enhance α-secretase activation in human fibroblasts, reduce $A\beta_{1–42}$ levels, and reduce mortality of transgenic mice [59]. In a human fibroblast cellular model of AD, treatment with $A\beta_{1–40}$ or $A\beta_{1–42}$ produced AD-specific deficits of PKC and ERK1/2 phosphorylation [13,16] that were reversed by bryostatin. Thus, bryostatin shows the potential of treating both the symptoms and the underlying neurodegeneration of AD. Bryostatin, unlike phorbol esters, is not tumorigenic and has been tested in over 1200 patients, with minor toxicity for lower dosage regimens. Bryostatin was originally used because of its ability to downregulate PKC. The tumor-promoting characteristics of phorbol ester TPA (12-O-tetradecanoylphorbol-13-acetate) in fact provided a rationale for seeking agents that blocked or downregulated PKC.

The biochemical endpoints for the effects of bryostatin on PKC in memory and AD depend on activation, whereas in antitumorigenic therapies the biochemical endpoints have focused on PKC downregulation and thus inhibition. Bryostatin-induced PKC activation is transient, lasting ~30 min and is immediately followed by ubiquitin-proteasome-mediated downregulation (lasting hours) and then increased *de novo* synthesis of PKC (Table 1).

The dosing regimens optimized for PKC activation and *de novo* PKC synthesis do not involve the higher concentrations of bryostatin or prolonged durations of exposure used in the phase II cancer studies. These same lower-dose regimens have also been found [60] to induce enhancement of the number and dimension of post- and presynaptic structures in hippocampi treated with *in vitro* and *in vivo* dosing regimens.

The multiple effects of PKC isozymes in other signaling cascades, such as those involved in pain, diabetes [61] and cardiac physiology, provide a challenge for using PKC activation therapy for AD. Repeated low-dose bryostatin activation of PKC should reduce toxicity, and adjuvants that antagonize the effects of bryostatin on PKC in non-CNS organ systems (e.g. cardiac tissues and peripheral vasculature) should also help minimize toxic side effects outside the targeted brain tissues. The demonstrated preclinical efficacy of bryostatin to enhance memory and reduce neurodegeneration provides additional compelling evidence that AD could arise from PKC signaling deficits common to memory and AD pathophysiology pathways.

lowering the levels of β-amyloid. Like Akt/PKB, PKC also inhibits glycogen synthase kinase-3β (GSK-3β), which is one of the principal enzymes for phosphorylating tau. The Wnt pathway also indirectly inhibits GSK-3β. Thus, PKC could act on both the amyloid and tau pathways in AD pathology. The calcium-binding protein calsenilin, which is homologous to the learning-associated protein calexcitin, interacts with presenilin and modulates K^+ channel activity. Presenilin is part of the γ-secretase complex that cleaves APP. Presenilin might also be essential for PKC to activate α-secretase; however, the mechanism of this effect is unclear. Because ERK1/2 and TACE co-precipitate, and ERK1/2 phosphorylates TACE at Thr735, ERK1/2 could directly regulate TACE and perhaps other ADAM family members (e.g. ADAM-9 and -10) [37]. These MAPK pathways typically comprise these elements: MAPK; MEK, which phosphorylates and activates MAPK; and MEK kinase, which activates MEK [38]. Whereas PKCδ can phosphorylate ADAM-9 directly, ADAM-17 (TACE) is phosphorylated by ERK1/2. However, TACE also contains numerous potential PKC phosphorylation sites, suggesting the possibility of direct involvement of PKC. TACE cleaves APP at the α-secretase site *in vitro* and this cleavage is inhibited by tumor necrosis factor α protease inhibitor (TAPI) and Immunex compound-3 (IC-3). Fibroblasts obtained from TACE knockout mice produce lower levels of sAPPα and do not show regulated sAPPα secretion. This and other findings implicate another disintegrin metalloproteinase, ADAM-10, in cleaving APP at the α-secretase site, in addition to a constitutively active α-secretase activity that is PKC independent. α-Secretase cleaves APP at position 16 within the Aβ domain, producing sAPPα (the ectodomain of APP terminating at the α-secretase cleavage site) and C83 (the 83-amino acid terminal segment of APP). Cleavage of C83 generates a 3-kDa fragment that is thought to be nonamyloidogenic. APP cleavage by α-secretase can occur at the plasma membrane, but PKC-regulated α-secretases also reside in the trans-Golgi network (TGN), which is also a major site for β-secretase activity. Because most of secreted Aβ is likely to be produced in the TGN, whereas intracellular $A\beta_{1–42}$ is produced in the endoplasmic reticulum (ER) intermediate compartment (IC), both β and α-secretase are apparently active in both ER-IC and TGN compartments, and at the plasma membrane. **(b)** Overview of the pathways in AD and learning and memory that involve PKC. PKC can be activated by a variety of factors, including pathways leading from fibroblast growth factor 18 (FGF-18), insulin growth factor (IGF) and phospholipase C (PLC). Other factors, such as β-amyloid, reduce PKC activity. PKC can affect DNA transcription by its effects on the RNA-binding protein ELAV, mitogen-activated protein kinase (MAPK) or transcription factors such as NF-κB. PKC also activates α-secretase directly, and indirectly through MAPK, which reduces levels of amyloid plaques. PKC inhibits GSK-3β, thereby potentially reducing the levels of hyperphosphorylated tau, the major component of neurofibrillary tangles. Abbreviation: Cdk5, cyclin-dependent kinase 5.

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