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Pharmacology of protein kinase C activators: Cognition-enhancing and antidementic therapeutics

Miao-Kun Sun *, Daniel L. Alkon

Blanchette Rockefeller Neurosciences Institute, 9601 Medical Center Drive, Academic and Research Building, The 3rd floor, Room 319, Rockville, MD 20850, USA

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ABSTRACT

Evidence is accumulating indicating that some protein kinase C (PKC) isozymes play an essential role in various phases as well as types of learning and memory. Abnormal functions of PKC signal cascades in the brains have been found to represent one of the earliest changes in patients with Alzheimer's disease (AD) and other types of memory deficits, including those related to cerebral ischemic/stroke events. In preclinical studies, an inhibition or impairment of PKC activity leads to compromised learning and memory, whereas an appropriate activation of some PKC isozymes results in an enhancement of learning and memory and/or antidementic effects against memory disorders. PKC activators not only increase activity of PKC isozymes and thereby restore PKC signaling activity, including neurotrophic activity, synaptic/structural remodeling, and synaptogenesis in the hippocampus and related cortical areas, but also reduce the accumulation of neurotoxic amyloid and tau protein hyperphosphorylation in the brain. These observations strongly suggest that PKC isoform pharmacology may represent an attractive area for the development of cognition-enhancing agents and therapeutics against memory loss in the future.

Contents

1. Introduction

Protein kinase C (PKC) isoforms are monomeric polypeptides, consisting of a multigene family of phospholipids-dependent, serine–threonine kinases. PKC is central to many signal transduction pathways. It is ubiquitously and densely expressed in the brain (Saito et al., 1988) and activated by Ca^{2+}, phospholipids and diacylglycerol, phorbolesters or other PKC activators. Activation of PKC isozymes leads to phosphorylation of a variety of target proteins, depending on the cell types and isoforms involved. Phosphorylation of the hydroxyl moiety of serine and threonine of a given protein often alters its stability, protein–protein interactions, cellular distribution, and catalytic activity, allowing the cell to transmit signals from the plasma membrane to their molecular targets as well as to the nucleus. One of the phosphoproteins, for

Abbreviations: Aβ, amyloid-β peptide; AD, Alzheimer's disease; AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazoleproprionate; aPKC, atypical PKC; APP, amyloid precursor protein; ARE, adenine- and uridine-rich element; cPKC, classical PKC; DCP-LA, 8-[2-(2-Pentylcyclopropyl-methyl)-cyclopropyl]-octanoic acid; DGK, diacylglycerol kinase; ELAV, embryonic lethal abnormal vision; ERK, extracellular signal-regulated kinase; GABA, Γ-aminobutyric acid; GAP-43, growth-associated protein 43; GluR, glutamate receptor; GSK, glycosynthetase kinase; H-7, 1-(5-isoquinolinesulfonyl)-2-methyl-piperazine; 5-HT, serotonin; LTD, long-term depression; LTP, long-term potentiation; mGluR, metabotropic GluR; nPKC MAPK, mitogen activated protein kinase; MARCKS, myristoylated alanine-rich C-kinase substrate; MEK, MAP kinase kinase; NCAM, neural cell adhesion molecule; NMDA, *N*-methyl-D-aspartate; NMDAR, NMDA receptor; NR, NMDA receptor; nPKC, novel PKC; PDZ, postsynaptic density zone; PICK, protein interacting with C kinase; PI3K, phosphoinositide 3-kinase; PKA, phosphokinase A; PKC, protein kinase C; PKM, persistently active kinase; RACK, receptor for activated C-kinase.

* Corresponding author. Tel.: 301 294 7181; fax: 301 294 7007.
E-mail address: mksun@brni-jhu.org (M.-K. Sun).

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instance, is GAP-43, a growth-associated protein with an approximate molecular weight of 43 kDa. PKC isozymes are thus involved in the modulation of neurite outgrowth/neuronal plasticity, synaptic functions/transmission, functions of membrane proteins, including enzymes and channels, metabolism, inflammation, carcinogenesis, neuronal proliferation, gene expression regulation, neuroprotection, neurodegeneration, neurogenesis, behaviors, and cognition including learning and memory (Alkon & Rasmussen, 1988; Hama et al., 2004; Rossi et al., 2005). In addition, PKC signaling cascades become abnormal in disease progression and may underlie pathogenesis of some disorders. Agents that act on the PKC signaling cascades are therefore not only useful for the investigation of cellular functions but also have great potential as therapeutic pharmaceutics. The focus of this review is, however, the potential of PKC agents as antidementic and cognition-enhancing therapeutics.

2. Protein kinase C isozymes

There are twelve PKC isoforms that have currently been identified in the mammals. The number of isoforms differs in other species. For instance, at least three isoforms have been found in *Aplysia*, namely Apls I, II, and III (Sossin, 2007).

Based on their homology and sensitivity to activators, the twelve isozymes in the mammals are commonly divided into 3 subfamilies: classical PKC (cPKC), novel PKC (nPKC), and atypical PKC (aPKC). The cPKC isoforms (α, β_I, β_{II}, and γ) contain four homologous domains (C1, C2, C3, and C4), which are interspaced with isozyme-unique (variable or V) regions and require Ca^{2+}, phosphatidylserine, diacylglycerol, or other PKC activators for activation. The two main regulatory domains (the activator-binding C1 and the Ca^{2+}-binding C2 domains) are at the NH_2-terminal, mediating membrane association and activation. The co-existence of an increased Ca^{2+} concentrations is required for a cPKC activation. A pseudosubstrate sequence (see below) is also located adjacent to the C1 domain. The C2 domain also contains binding sites for lipids and proteins. A C-terminal active site, on the other hand, contains the C3 and C4 domains, functioning as a serine/threonine kinase. The C3 region possesses the binding site for ATP, the phosphate donor for phosphotransferase activity, while the C4 region has the binding site for the substrates. The nPKC isoforms (δ, ε, ε', η, θ, and μ) lack the C2 homologous domain and do not require Ca^{2+} for activation. The aPKC isoforms (ζ and λ/ι), on the other hand, lack both the C2 and one half of the C1 homologous domains and are insensitive to Ca^{2+}, diacylglycerol, phorbol esters or other PKC activators. aPKC isozymes can, however, be activated by phosphatidyl-serine, arachidonic acid and ceramide.

The PKC isoforms, except PKCμ (human) and its murine homologue, PKD, contain an N-terminal pseudo-substrate motif, an autoinhibitory domain, near their C1 domains. This motif binds to its catalytic region, thereby keeping the enzyme inactive. PKC isoforms can thus be activated through proteolytic cleavage of this regulatory fragment, resulting in transformation into a persistently active kinase (called PKM). PKCδ, for example, can be cleaved by caspase-3 to generate a catalytically active fragment (Emoto et al., 1995; Kanthasamy et al., 2003), a response that leads to dopaminergic degeneration induced by dieldrin, a potential environmental risk factor for development of Parkinson's disease (Kitazawa et al., 2003), since PKCδ-specific inhibitor and caspase-3 inhibitor eliminate dieldrin-induced apoptosis of the neurons.

Binding of PKC activators to the isoforms leads to a dissociation of the pseudo-substrate from their catalytic regions. It has been well established that activation of PKC isozymes involves their redistribution in the cells (translocation). The unique cellular functions of different PKC isoforms appear determined by their subcellular location. For instance, activated PKCβI is found inside the nucleus, while activated PKCβII, at the perinucleus and cell periphery of cardiac myocytes. The localization of different PKC isoforms to different areas of the cell appears due to binding of the activated isozymes to specific anchoring molecules, receptors for activated C-kinase (RACKs), which function by selectively anchoring activated PKC isoforms to their respective subcellular sites. RACKs bind only activated PKC isoforms but are not necessarily substrates of the enzyme. Nor is the binding to RACKs mediated via the catalytic region of the kinase. The binding is, however, required for PKC isoforms to produce their cellular responses. Evidence has been provided that inhibition of PKC binding to RACKs in vivo inhibits PKC translocation and PKC-mediated functions (Smith & Mochly-Rosen, 1992; Ron & Mochly-Rosen, 1995; Johnson et al., 1996). Peptides that mimic either the PKC-binding site on RACKs or the RACK-binding site on PKC isozymes are isozyme-specific translocation inhibitors of PKCs. For instance, an eight amino acid peptide derived from PKCε (εV1-2; Glu Ala Val Ser Leu Lys Pro Thr) contains a part of the RACK-binding site on PKCε and selectively inhibits PKCε-mediated functions in cardiac myocytes (Mochly-Rosen, 2000). Peptides that are able to inhibit or activate the translocation or function of PKCδ include those selected from δV1-1, δV1-2, and δV1-5 (Mochly-Rosen & Chen, 2005). The structural requirement for the isozyme-specific binding holds great interest for the development of PKC isozyme-selective non-peptide inhibitors and activators.

3. Protein kinase C signaling in memory and memory consolidation

It is now well established that PKC isoforms play an essential role in many types of learning and memory (Bank et al., 1988; Olds et al., 1989; Alkon et al., 2007; Nelson et al., 2008, 2009). PKC is activated by synaptic inputs and intracellular signals that are involved in information processing in cognition, including glutamatergic inputs (Hasham et al., 1997), cholinergic inputs (Chen et al., 2005), serotonergic inputs (Carr et al., 2002, 2003), dopaminergic inputs (Maurice et al., 2001), intracellular calcium and diacylglycerol elevations, and other hormones (Sato et al., 2004). PKC isoforms have been shown to regulate phosphorylation of various substrates, including the myristoylated alanine-rich C-kinase substrate (MARCKS), GAP-43, and the NMDA receptor, all of which are involved in information storage processes. Evidence has been provided that memory task learning is associated with PKC immunoreactivity in the principal hippocampal neurons (van der Zee et al., 1995) and stimulation of muscarinic cholinergic receptors is associated with an increase in PKCγ immunoreactivity (van der Zee et al., 1992). Changes in the activity of PKC's downstream signaling molecules are also involved. The expression of GAP-43 (Holahan & Routtenberg, 2008), for instance, is up-regulated during spatial learning and memory (Pascale et al., 2004). Transgenic mice overexpressing GAP-43, not beyond an optimum point (Rekart et al., 2004), have been found to exhibit an enhanced memory in a maze task (Routtenberg et al., 2000). Most homozygous GAP-43 knock-outs die soon after birth. Heterozygous GAP-43 knockout mice have impaired hippocampus-dependent memory, such as contextual fear conditioning, while showing a similar startle response to neutral tones of increasing intensity and a similar response and sensitivity to an incremental series of foot shocks as the wild-type (Chung et al., 2003; Rekart et al., 2005). In the sensory neurons of *Aplysia*, associative long-term facilitation of synapses, produced by a single pairing of a brief tetanus with 5-HT, requires a rapid PKC-dependent and rapamycin-sensitive increase in local sensorin synthesis and secretion (Hu et al., 2007), responses that probably involve PKC Apl I (Sossin, 2007).

3.1. Synaptic transmission and neuronal functions

Plasticity of neuronal and synaptic connections is believed to play a critical role in information processing. PKC regulates the synthesis, vesicle-refilling, and release of many neurotransmitters, including the cholinergic, the γ-aminobutyric acid (GABA)-ergic, and the

glutamatergic systems (Malenka et al., 1986; Nicholls, 1998; Stevens & Sullivan, 1998; Dobransky et al., 2004; Okada et al., 2004) as well as gene expression in mature neurons (Roberson et al., 1999). In the neural networks, activation of PKC isoforms generally facilitates synaptic plasticity, including such responses as increases in Ca^{2+} influx and neurotransmitter release, a decrease in a Ca^{2+}-activated K current in the hippocampus, actions that result in an enhancement of neuronal excitability and potentiation of synaptic responses (Alkon et al., 1986; Farley & Auerbach, 1986; Bank et al., 1988; LoTurco et al., 1988; Alkon et al., 1998; Zhang et al., 2005; Cohen-Matsliah et al., 2007).

Synaptic plasticity and functions can also be regulated by modulating the organization of the synaptic cytoskeleton. Synapses are polarized structures in which proteins and mRNA become asymmetrically localized. Many synaptic receptors are anchored to the actin submembrane matrix. PKC isoforms are known to regulate the activity and cell surface expression of several plasma membrane proteins, including G-protein coupled receptors, neurotransmitter transporters (serotonin, dopamine, norepinephrine, glutamate, and GABA), and the Na^+/H^+ antiporter. Activation of PKC also increases the activity and cell surface expression of the neuronal glutamate transporter EAAC1 (González et al., 2002), which is enriched in the pyramidal cells of the cortex and hippocampus. The *N*-methyl-D-aspartate receptors (NMDARs), for instance, bind α-actinin, an actin binding protein (Wyszynski et al., 1997). At *Drosophila* glutamatergic presynaptic structures, aPKC regulates the stability of microtubule by promoting the association of the MAP1B-related protein Futsch to the microtubules, while at the post-synaptic structures, it regulates the synaptic cytoskeleton by controlling the extent of actin-rich and microtubule-rich areas (Ruiz-Canada et al., 2004).

3.1.1. Glutamatergic system

Glutamate, the major excitatory transmitter in the brain, is critical to the control of behaviors and cognitive ability. The glutamatergic system interplays with PKC cascades in signal transduction. In the cultured cerebellar granule neurons, for instance, NMDAR activity has been shown to regulate PKC activity (Wang et al., 2004). On the other hand, PKC mediates (−)-epigallocatechin gallate, the main polyphenolic constituent of green tea-induced Ca^{2+}-dependent glutamate release in rat cerebral cortex (Chou et al., 2007). PKC also mediates brain-derived neurotrophic factor-mediated modulation of NMDAR subunit 1 in the dorsal horn of rat spinal cord (Salck et al., 2004). The α-amino-3-hydroxy-5-methyl-4-isoxazoleproprionate (AMPA) receptor subunits glutamate receptor (GluR)1 and GluR2 contain type I and type II postsynaptic density protein of 95 kDa/Discs-large/ZO-1 (PDZ) binding motifs, respectively, and so does the metabotropic GluR (mGluR)7α. The C terminus of PKCα has a type I PDZ binding motif, where GluR2 has a type II PDZ binding motif. Both motifs are recognized by the PDZ domain of protein interacting with C kinase 1 (PICK1). The PDZ domain of PICK1 appears to have distinct PKCα and GluR2 binding subsites and PICK1-PKCα-controlled phosphorylation regulates the synaptic expression and function of GluR2 (Dev et al., 2004). Knock-in mice lacking the PDZ-ligand motif of mGluR7α show an impaired PKC-dependent regulation of glutamate release and deficit in spatial working memory (Zhang et al., 2008).

Changes in PKC activity affect both the inotropic and metabotropic GluRs. Activation of PKC leads to phosphorylation of GluR 2/3 (at serine 880) in the Purkinje cells. The GluR 2/3 phosphorylation appears to be the critical step for parallel fiber long-term depression (LTD) (Rekart et al., 2005). Phosphorylation by PKC of GluR1 at its serine 818 residue, on the other hand, controls synaptic incorporation of GluR1-containing AMPA receptors into the synapses during LTP (Boehm et al., 2006). PKC activation has also been shown to mediate AMPA receptor subtype switch (from GluR2-lacking [Ca^{2+}-permeable] to GluR2-containing [Ca^{2+}-impermeable] receptors), produced by an activation of the extrasynaptic NMDA receptors in mouse cerebellar stellate cells (Sun & Liu, 2007). In the perirhinal cortex, mGluR-LTD requires an activation of the PKC–PICK1 signaling pathway (Jo et al., 2008). In the hippocampal CA1 pyramidal neurons, mGluR6-containing kainate receptors are probably involved in the PKC-mediated inhibition of the slow after-hyperpolarization (Melyan et al., 2002). Glutamate also desensitizes mGluR5a and mGluR5b, through PKC-mediated phosphorylation of mGluR5 at multiple sites (Gereau & Heinemann, 1998). In the pyramidal neurons of rat prefrontal cortex, mGluR activity has been shown to enhance NMDAR currents via a PKC-dependent mechanism (Tyszkiewcz et al., 2003). mGluRs couple to G-protein and activate phospholipase. Their activation results in the hydrolysis of membrane phosphatidylinositol bisphosphate to inositol trisphosphate and diacylglycerol, which activates PKCs. The activation involves protein–protein interaction at the receptor and signal pathway levels. Homer proteins, the products of neuronal immediate early genes, selectively bind to the carboxy-termini of certain cell-surface receptors, intracellular receptors, and binding proteins, and may be involved in PKC activation.

3.1.2. GABAergic system

In the adult mammalian central nervous system, GABA is the major inhibitory neurotransmitter. In addition to the agents that act on GABA receptors (GABARs) as agonists or antagonists, GABAR currents can also be modulated by positive and negative allosteric agents, such as benzodiazepines, barbiturates, neurosteriods, and zinc. PKC isoforms can phosphorylate several GABAR subunits at their major intracellular domains, changing GABARs' functions and their allosteric modulations. Activation of PKCs decreases GABAR function in most cases (Leidenheimer et al., 1993; Krishek et al., 1994; Filippova et al., 2000) or increases GABAR currents in some cases (Lin et al., 1994, 1996; Poisbeau et al., 1999). In the hippocampus, PKC activation has been reported to increase miniature inhibitory synaptic current (mIPSC) peak amplitudes of the granule cells but to have no effect on the mIPSC in the CA1 neurons (Poisbeau et al., 1999). In the NT2-N neurons, an activation of PKC isozymes has been shown to result in a reduced apparent affinity of diazepam to the GABARs and a decreased allosteric enhancement by benzodiazepines (Gao & Greenfield, 2005).

Effects of PKC activation on the GABAergic transmission may thus depend on subunit composition and cell-specific factors. PKCε, for example, acts as a selective modulator of endogenous allosteric agonists of $GABA_A$ receptors. Compounds that inhibit PKCε act in synergy with drugs acting on $GABA_A$ receptors and may have effects on anxiety, alcohol consumption, self-administration of other drugs of abuse, either alone or in conjunction with allosteric agonists of the $GABA_A$ receptors. These compounds, therefore, may have potential therapeutic values in treating anxiety, addiction, withdrawal syndrome, skeletal muscle spasms, convulsive seizures, and epilepsy. Mice with PKCε deficits exhibit less fear and anxiety and are hypersensitive to the sedative-hypnotic effects of compounds acting at the $GABA_A$ receptors (such as ethanol, pentobarbital, or benzodiazepine) than the wild-type mice.

3.1.3. Cholinergic system

It is well established that the cholinergic system in the central nervous system plays an essential role in learning and memory and other brain functions. Functional deficits in the brain cholinergic system and neuronal injury are among the earliest deficits in AD. Functional interaction between the cholinergic system and PKC cascades has been reported frequently. Muscarinic activation stimulates, through G-protein coupled receptors, phospholipase C, which cleaves the membrane phospholipids phosphatidyl-inositol-4,5-bisphosphate to inositol-1,4,5-trisphosphate (IP_3) and diacylglycerol, a cPKC and nPKC activator. IP_3 initiates Ca^{2+} release from intracellular stores. High Ca^{2+} levels are required for activation of cPKCs. PKC activation, on the other hand, enhances acetylcholine

release from rat hippocampal slices (Chaki et al., 1994). Activation of the pre-synaptic α7 acetylcholine receptor on the glutamatergic terminals in the CA1 region of the intact rat hippocampus facilitates glutamate release via an action on PKC (Yamamoto et al., 2005). The action of PKC-induced pre-synaptic facilitation may thus have therapeutic values in antidementic treatment (Nishizaki et al., 2000).

It has also been shown that arachidonic acid stimulates choline acetyltransferase activity through PKC activation in cultured spinal cord neurons (Chalimoniuk et al., 2004). Choline acetyltransfase phosphorylation in neurons is mediated predominantly by PKC at Ser-476 (required for phosphorylation by PKC at other serine residues to proceed), with PKC activation increasing phosphorylation at Ser-440 and enhancing choline acetyltransferase activity (Dobransky et al., 2004).

3.1.4. Dopaminergic system

There is mutual interaction between the dopaminergic system and PKC. It has been shown that activation of PKC isoforms results in rapid degradation of dopamine transporter ($t_{1/2}$ about 1–2 h) in both porcine aortic endothelial and HeLa cells (Miranda et al., 2005), through an accelerated internalization and probably lysosomal degradation. In the C6 glioma cells, the former is mediated by PKCε, while the latter, PKCα through PI3K (Davis et al., 1998; González et al., 2002). The dopamine-mediated enhancement of spike firing in nucleus accumbens shell medium spiny neurons can be prevented by the PKC inhibitor bisindolymaleimide but not by the phospholipase C inhibitor 1-[6-((17b-3-methoxyestra-1,3,5(10)-trien-17-yl) amino) hexyl]-1H-pyrrole-2,5-dione, suggesting a role for the diacylglycerol-independent aPKCs (Hopf et al., 2005). The aPKC-mediated dopaminergic enhancement of spike firing in the nucleus accumbens shell may play a critical role in related goal-directed behavior.

3.2. K channels

Functional operation of K channels plays an important role in neuronal function as well as has a great impact on synaptic transmission (Alkon et al., 1982). All shaker K channels contain PKC sites, whose phosphorylation down-regulates K channel activity. The voltage-gated K channel protein Kv1.3, which is expressed in the central nervous system, for instance, has been implicated as insulin receptor substrate (Fadool et al., 2000). Inhibition of Kv1.1 K channel expression with antisense impairs associative memory in mice and rats (Meiri et al., 1997). Inhibiting Kv1.3 activity in rats increases associative learning and memory (Kourrich et al., 2001) and may mediate decreased food intake, weight loss, decreased body fat, and increased glucose uptake. PKC also down-regulates ATP-sensitive potassium (K_{ATP}) channel number, via a K_{ATP} internalization (Hu et al., 2003). K_{ATP} is activated by metabolic stress, such as hypoxia, cerebral ischemia, and metabolic inhibition, to protect neurons. Excessive channel activation, however, may have deleterious consequences in information processing and storage, such as silencing neurons and networks.

3.3. Axon regeneration and synaptogenesis

Functional operation of PKC isoforms is essential in the regulation of neural cell proliferation, contraction and survival (Maher, 2001). PKC inhibitors have been shown to block neurite outgrowth in the retinal axons (Heacock & Agranoff, 1997), the dorsal root ganglion neurons (Theodore et al., 1995), the sympathetic neurons (Campenot et al., 1994), the PC12 cells (Kolkova et al., 2000), and the hippocampal organotypic cultures (Toni et al., 1997), or to promote dendritic growth in Purkinje cells in the cerebellar slice culture (Metzger & Kapfhammer, 2000) and extension of dorsal root ganglion cell filopodia (Bonsall & Rehder, 1999). The majority of the inhibitory activity for axonal regeneration in the local environments of the central nervous system is associated with components of myelin and molecules in the glial scar at the lesion site, mediated by cPKC activity (Sivasankaran et al., 2004). Inhibiting PKC activity has thus been found to block the inhibitory activities of myelin and its components of the central nervous system and to stimulate neurite outgrowth in the presence of myelin both in vitro and in vivo. The mechanism of action may involve an inhibition of the growth inhibition by endogenous, myelin growth repulsion factors.

Activity of some PKC isoforms is critically involved in synaptic remodeling, rearrangement, and regeneration. Activation of PKC with 12-myristate 13-acetate, an analogue of diacylglycerol, induces rapid morphological plasticity, formation of dendritic lamellae, in dendrites of cultured hippocampal neurons, a response via the small GTPases Rac and Rho-dependent mechanisms but not extracellular signal-regulated protein kinase (ERK; Pilpel & Segal, 2004). Lamellar formation involves actin polymerization and may reflect synaptic rebuilding and rearrangement. Rac and Rho inactivation, however, has no apparent electrophysiological effects on activating PKC. Recent evidence suggests that astrocytes are active participants in formation and modification of synapses (Haydon, 2001). Local astrocytic contact of cultured rat hippocampal neurons via integrin receptors promotes global synaptogenesis (Hama et al., 2004). The contact activates PKC through arachidonic acid cascade in neurons, triggering excitatory synaptogenesis (Hama et al., 2004). The process can be blocked by inhibitors of both integrins and PKC (hama et al., 2004). It is not clear, however, whether the integrin-PKC-cascade plays an essential role in the formation of memory traces in adult brains. Activation of PKC with bryostatin-1 has also been found to enhance synaptogenesis and synaptic remodeling (Hongpaisan & Alkon, 2007a), responses associated with improved performance in spatial learning and memory tasks in rats (Sun & Alkon, 2005). It is important to emphasize here that long-term and selective maintenance of dendritic spines is associated with lasting memories (Yang et al., 2009; Xu et al., 2009).

3.4. Neuronal survival and death

The switch between neuronal survival and death is determined by several factors and cascades in disorders and aging. Different PKC isozymes have been shown to influence the process of neurite outgrowth or the induction of apoptosis, depending on the cell type and apoptotic signal. PKCα, β, ε, and ζ can function as suppressors of apoptosis, whereas PKCδ and θ are pro-apoptotic in function. In the neuroblastoma cells, for instance, PKCε induces neurite out-growth, whereas PKCδ and PKCθ evoke apoptosis. Stimulation by the neural cell adhesion molecule-mimetic peptide, C3d, elicits phosphorylation of PKC in primary cerebellar granular neurons, probably using PKCε as a common downstream mediator (Kolkova et al., 2005). PKCε can also induce neurite outgrowth independent of its catalytic activity via a region encompassing its C1 domains. Both C1a and C1b are important for neurite induction. Further studies reveal that only 4 amino acids N-terminal and 20 residues C-terminal of the C1 domains are necessary for neurite induction (Ling et al., 2005). Evidence has also been provided that PKC may play an important role in the survival of the spiral ganglion neurons. After deafferentation, activation of the PKCβ1 with either phorbol esters or bryostatin-1 induces survival and neurite regrowth and rescues the spiral ganglion neurons from cell death (Lallemend et al., 2005), responses probably mediated by recruiting both the mitogen-activated protein kinase kinase (MEK)/ERK pathway and phosphoinositide 3-kinase (PI3K)/Akt pathway (Lallemend et al., 2005).

An interaction between neurotrophic factors and PKC isoforms represents an important endogenous mechanism in molecular switch between survival and death. PKC isozymes may be activated by activity of nerve growth factor through two pathways. Nerve growth factor can influence PKC activity through the phosphorylation of the activation loop of PKC by phosphoinositide-dependent kinase-1 (Toker, 2000; Parekh et al., 2000). Nerve growth factor activates phospholipase C-γ,

which, upon binding to phosphorylated Tyr^{785} in Trka, is itself phosphorylated and activated, hydrolyzing phosphatidylinositol 4,5-biphosphate to produce diacylgly-cerol and inositol trisphosphate and thus activating PKC. Nerve growth factor activates phosphatidylinositol 3-kinase and PKC in sympathetic neurons and PKC activation rescues neurons from apoptosis induced by the withdrawal of nerve growth factor (Favit et al., 1998; Pierchla et al., 2004). PKC may also mediate neuroprotective effects of estrogen and protect neurons against Aβ neurotoxicity (Cordey et al., 2003). There is a direct neuroprotective effect of PKC against Aβ since the protection is against added $Aβ_{42}$ (25 μM, 24 h) in culture and is blocked by pharmacological inhibitors of PKC (Cordey et al., 2003). Estrogen activates cPKC and/or nPKC in a variety of cell types non-genomically and can translocate PKCγ, through the G-protein coupled estrogen receptors (Qiu et al., 2003).

In rats, kainic acid administration induces upregulation of PKCδ mRNA and protein in the cortex and hippocampus (Kaasinne et al., 2002). Kainate at 50 μM induces PKCδ translocation from the soluble to the particulate fraction in cultured cortical neurons obtained from mice, as early as 15 min following kainite exposure (Jung et al., 2005). Inhibition of PKCδ with rottlerin significantly increases kainite-induced neuronal death, while phorbol 12-myristate 13-acetate attenuates the kainite-induced neuronal death (Nitti et al., 2005), suggesting a protective role of PKCδ against kainite toxicity. On the other hand, PKCδ has been found to mediate glycoxidation-dependent apoptosis in the NT2 human neurons, since rottlerin is able to protect neurons from the glycoxidation-dependent apoptosis (Nitti et al., 2005). Nuclear translocation of PKCζ, a predominantly cytosolic enzyme, is sensitive to caspase-3 inhibition and is believed to mediate NMDA-induced death of the cortical neurons. The nuclear translocation of PKCζ induced by NMDA involves caspase-3-dependent PKCζ degradation, generating a fragment of about 50 kDa. Like other aPKC isozymes, PKCζ is not activated by Ca^{2+}, diacylglycerol, phorbol esters or bryostatins. It is activated by several lipid mediators, including phosphatidic acid, phosphatidylinositol 3,4,5-triphosphate, arachidonic acid and ceramide. Aspirin directly inhibits PKCζ activity, protecting the NMDA-induced death of the cortical neurons (Crisanti et al., 2005). On the other hand, evidence is available that PKCζ is necessary and sufficient for persistence of memory in *Drosophila* (Hernandez et al., 2003).

The impact of sensitivity of PKC isoforms to reactive oxygen species needs further evaluation. PKC isoforms are activated by reactive oxygen species. PKCε, for instance, has been proposed to mediate reactive oxygen species-triggered death of the cortical neurons (Jung et al., 2004). PKCδ has been shown to be a key downstream mediator of manganese-induced mitochondrial-dependent apoptosis in mesencephalic dopaminergic neurons via caspase-3 activation (Latchoumycandane et al., 2005). Proteolytic activation of PKCδ by caspase-3, probably an isoform-specific event, plays an important role in the apoptotic cell death of the dopaminergic cells (Kanthasamy et al., 2003). Manganese exposure causes manganism, a neurological disorder similar to Parkinson's disease. The N27 cells expressing a catalytically inactive $PKCδ^{K376R}$ protein (PKCδ dominant negative mutant) or a caspase cleavage resistant $PKCδ^{D327A}$ protein (PKCδ cleavage resistant mutant) are resistant to manganese-induced apoptotic cell death (Latchoumycandane et al., 2005).

In short, PKC activation is critically involved in the formation of many types of memories, consistent with the evidence that PKC activators enhance spatial learning and memory in rats (Sun & Alkon 2005; Hongpaisan & Alkon, 2007a; Sun & Alkon, 2008), a response sensitive to co-administration of 1-(5-isoquinolinesulfonyl)-2-methyl-piperazine (H-7), a PKC inhibitor (Sun & Alkon 2005).

4. Protein kinase C dysfunction and dementia

Several pieces of evidence indicate an essential role of PKC signaling in memory formation (Rossi et al., 2005; Amadio et al., 2006; Alkon et al., 2007). PKC isozymes are important signaling molecules in learning and memory (Bank et al., 1988; Alkon et al., 1998, 2007; Amadio et al., 2006; Lorenzetti et al., 2008; Nelson et al., 2008; Sacktor, 2008; Serrano et al., 2008), including spatial learning and memory (Olds et al., 1989, 1990; Paylor et al., 1991, 1992; Colombo et al., 1997; Vázquez & de Ortiz, 2004; Sun & Alkon, 2008) and consolidation of spatial memory (Bonini et al., 2007), learning and memory of eye blink conditioning (Bank et al., 1988; Schreurs et al., 1996, 1997; Van der Zee et al., 1997; Alkon et al., 1998; Wang et al., 2008), olfactory discrimination learning (Olds et al., 1994), conditioned taste aversion (Yasoshima and Yamamoto, 1997; Nunez-Jaramillo et al., 2007), contextual fear memory (Ahi et al., 2004; Levenson et al., 2004), and conditioned avoidance (Jerusalinsky et al., 1994). Impaired learning and memory occurs when PKC cascades are interrupted in these memory tasks.

Inhibition and impairment of PKC functions in majority cases lead to deficits in learning and memory. Intracerebroventricular injection of PKC inhibitors, for example, causes marked memory impairment in passive avoidance task and water maze task (Takashima et al., 1991). One exception is the report that curcumin-induced PKCδ degradation is associated with enhanced spatial learning in adult and aged rats (Conboy et al., 2009). Curcumin increases PKCδ degradation and neural cell adhesion molecule (NCAM) expression, probably through an increased phosphorylation of Tyr^{311} residue within the hinge region between the regulatory and catalytic regions of the isoform, resulting in an conformational change that makes the hinge region accessible to proteolytic cleavage (Kishimoto et al., 1989). The Tyr^{311} residue of PKCδ is flanked by a sequence that forms an optimal binding substrate for the Src family of kinases that constitutively complex with PKCδ but not other PKC isoforms such as PKCα or PKCε (Steinberg, 2004). Consistent with the observation is also the evidence that PKCδ expression in the brain can also be reduced by α-tocopherol (Zingg & Azzi, 2004) or environmental enrichment (Gallagher et al., 2001).

Functional deficits of PKC isoforms may underlie an impaired cognition experimentally and clinically. In mice with a deficit in PKCβ, learning of both cued and contextual fear conditioning is impaired although brain anatomy and hippocampal synaptic transmission, paired-pulse facilitation, and long-term potentiation of synaptic responses are all normal (Weeber et al., 2000). Expression of PKC isoforms and their functions, especially those in the hippocampus and related brain structures, are plastic and vulnerable to various factors, including stress and neurotoxic amyloid. PKC dysfunction occurs in neurodegenerative disorders including AD (Cole et al., 1988; Govoni et al., 1993; Wang et al., 1994), leading to cognitive impairments. AD, for instance, is characterized by a devastating and progressive decline of memory and other cognitive functions, a disorder whose pathology is believed by many can be characterized by amyloid-β-peptide deposits and hyperphosphorylated tau. The ability to form new memory is especially impaired in AD. Aβ occurs in two predominant forms with different COOH-termini, Aβ40 and Aβ42, through cleavage of β-amyloid precursor protein (APP) by β-secretase(s) and γ-secretases. β-Secretase cleaves APP at the NH_2-terminus of APP, releasing a soluble NH_2-terminal fragment about 100-kDa (APPsβ) and a 12-kDa membrane-bound C99 fragment. PKC signaling pathway is impaired in AD, consistent with the evidence that Aβ reduces PKC isozyme levels (Wang et al., 1994; Desdouits et al., 1996; Pakaski et al., 2002). Aβ contains a putative PKC pseudosubstrate domain and can directly inhibit PKC activation, including PKCα and PKCε (Lee et al., 2004). Aβ treatment at 1 μM for 1 h induces such an inhibition, lasting for several hours (Lee et al., 2004). Through its binding to PKC, Aβ blocks PKC activation and induces PKC degradation (Cordey et al., 2003), reduces PKC-mediated phosphorylation (Chauhan et al., 1991; Govoni et al., 1993), and decreases PKC membrane translocation (Pakaski et al., 2002). This mechanism of action suggests that the type of interaction between PKC and Aβ would affect all the PKC isozymes that contain the pseudosubstrate binding site and that the soluble form of Aβ, including its

oligomers, would be pathologically most active. Aβ40, for instance, has been shown to induce translocation of PKC from membrane fraction to cytosol in cultured endothelial cells (Pakaski et al., 2002). Cleavage of APP by α-secretase, on the other hand, produces a large soluble fragment and a 10-kDa membrane-bound C83 fragment. C99 and C83 can be further cleaved by one or more γ-secretases, resulting in Aβ and a nonpathological p3 peptide, respectively. Tau is a microtubule-associated protein, typically found in the axon of neurons, involved in microtubule assembly and the stabilization of growth axons (Mailliot et al., 2000). Its hyperphosphorylation also prevents the binding of tau to taxol-stabilized microtubules and disrupts microtubule assembled from tau and tubulin (Mandelkow & Mandelkow, 1998). A number of protein kinases and protein phosphatases have been implicated in tau hyperphosphorylation, including glycosynthetase kinase 3β (GSK-3β), phosphokinase A (PKA), phosphokinase C and Src protein kinase.

5. Protein kinase C agents and memory

PKC isoforms are distributed in neuronal structures involved in a broad range of functions (Alkon et al., 1982; Brenner et al., 2004; Lee et al., 2006a; Pascale et al., 2007), regulating both the 'software' and the 'hardware' of the synapses. Activation of PKC isoforms potentiates synaptic responses in a variety of preparations (Alkon et al., 1986; Kaczmarek, 1987; Bank et al., 1988; LoTurco et al., 1988; Alkon et al., 1998; Stevens & Sullivan, 1998; Zhang et al., 2005). Activation of PKC leads to changes in such vital responses as enhancing Ca^{2+} action potentials, increasing neurotransmitter release, and decreasing voltage-gated Na^{+} currents (Carr et al., 2002, 2003; Chen et al., 2005, 2006) through enhancing intrinsic slow inactivation gating (Chen et al., 2006) and voltage-dependent K^{+} currents (Alkon et al., 1986; Farley & Auerbach, 1986) as well as Ca^{2+}-activated potassium current in the hippocampus, all relevant to information processing in cognition. Furthermore, PKC activation promotes synaptogenesis in the hippocampus (Hongpaisan & Alkon, 2007a). Activation of PKC isozymes to appropriate levels results in an enhancement of memory in general. Overactivation of PKC may, however, impair learning and memory, such as working memory in the young and old (Brennan et al., 2007). Interests have been raised to develop PKC activators as potential memory therapeutics, since it has been well established that PKC activators facilitate synaptic plasticity, enhance learning and memory, reduce neurotoxic amyloid production and accumulation, and inhibit tau phosphorylation.

There are several types of PKC activators, differing in chemical structures, isoform selectivity, and binding affinity. PKC activators, such as diacylglycerol, arachidonic acid, phorbol esters, bryostatins, aplysiatoxins, and teleocidins, bind to a hydrophilic cleft in a largely hydrophobic surface of the C1 domains. The binding results in an enhanced hydrophobicity of the surface and promotes the interaction between the C1 domain and the phospholipid bilayer of the cell membranes, driving removal of the pseudosubstrate region from the catalytic site of the enzyme. 8-[2-(2-Pentylcyclopropyl-methyl)-cyclopropyl]-octanoic acid (DCP-LA), on the other hand, has been shown to selectively activate PKCε, probably through binding to the phosphatidylserine binding site (Kanno et al., 2006; Nelson et al., 2009).

Diacylglycerol, an endogenous PKC activator, binds to the C1 domain of cPKC and nPKC. Its binding affinity for PKC isoforms is at μM levels (for displacing bound [20-3H]phorbol 12,13-dibutyrate from the sensitive PKC isoforms). The hydrocarbon chain is to facilitate partitioning into the lipid-rich membrane environment.

Phorbol esters have been commonly used to activate PKC isoforms experimentally. Phorbol esters, such as phorbol 12.13-dibutyrate and phorbol 12-myristate 13-acetate, also bind to the C1 domain of cPKC and nPKC, with binding affinities over 2 orders of magnitude greater than those of diacylglycerol. Their higher potencies come from a conformationally rigid orientation of hydrophilic pharmacophores. There are reports, however, that some observed effects that are produced with phorbol esters and viewed as PKC-mediated may involve other signaling molecules rather than PKCs.

Bryostatins are isolated from the marine *Bugula neritina* with chemical structure unrelated to phorbol esters. Bryostatin-1, a macrocyclic lactone, is an antineoplastic agent that potently activates PKC through binding to the C1 domain of cPKC and nPKC, with binding affinities at nM or sub-nM levels. There are numerous studies that indicate memory-enhancing properties of bryostatins. Bryosatin-1, for instance, improves learning and memories (Etcheberrigaray et al., 2004; Sun & Alkon, 2005). Several actions may underlie or contribute its memory-enhancing effects. PKC activation with bryostatin-1 not only facilitates synaptic functions/transmission but also induces the de novo synthesis of those proteins that are necessary and sufficient for subsequent long-term memory consolidation and enhances memory in *Hermissenda* (Alkon et al., 2005; Kuzirian et al., 2006) and *Lymnaea* (Rosenegger et al., 2008). The activator promotes stabilization of GAP-43 mRNA, resulting in an increased GAP-43 protein level as shown in human neuroblastoma cells (Pascale et al., 2005). The underlying mechanisms may also partially involve embryonic lethal abnormal vision (ELAV) proteins. ELAV proteins, or Hu antigens, are gene expression regulatory factors, binding to the adenine- and uridine-rich element (ARE) of the mRNA 3′ UTR, thus stabilizing and positively controlling gene expression. AREs exist in 5–8% of expressed genes in humans (Bakheet et al., 2003). In the human neuroblastoma SH-SY5Y cells, 15-min treatment with phorbol esters or bryostatin-1 upregulates neuron-specific ELAV proteins, such as HuB, HuC, and HuD, their colocalization with the translocated PKCα isozyme, and stabilization of GAP-43 mRNA, and increases GAP-43 expression (Pascale et al., 2005). In transgenic mice overexpressing HuD, paired-pulse facilitation of the mossy fiber to CA3 synapse at short inter-pulse intervals is increased but LTP is not altered (Tanner et al., 2008). The availability of bryostatins is limited by their low natural abundance and difficulty in total synthesis. The compounds contain a pharmacophoric region and a relatively lipophilic space domain. The latter can be modified, producing a variety of bryostatin analogs, many of which possess similar PKC-binding affinities as those of bryostatins.

6. Protein kinase C agents for the treatment of memory impairments and dementia

Therapeutic values of PKC activators on memory impairment and dementia lie on a combination of enhancing cognitive functions, facilitating neural and synaptic repairment/remodeling after injury and arresting some memory-impairing pathological cascades.

6.1. Alzheimer's disease and dementia

AD, a devastating and progressive decline of memory and other cognitive functions (e.g., a reduced ability to learn, loss of memory, decreased attention, judgment, and decision-making), robs the affected individuals of the quality of life. The main histopathological hallmarks of AD brain are extracellular senile plaques formed by Aβ deposits and intracellular neurofibrillary tangles consisting of paired helical filaments formed by hyperphosphorylated tau, at late stages of the disorder. Three pharmacological profiles favor potential use of bryostatin-1 and its analogs to restore cognitive dysfunction especially that associated with AD.

First, PKC isoforms are critically involved in signaling processing in learning and memory. Expression of some PKC isoforms decreases in aging (Cole et al., 1988) and neurotoxic Aβ inhibits/impairs PKC functions (Favit et al., 1998; Lee et al., 2004). Deficient functions of PKC isozymes may play a critical role in memory deficits in Alzheimer's disease. Functional restoration/facilitation of the PKC signal cascades thus represents a memory-enhancing mechanism.

Second, PKC, probably mediated by α and ε isoforms, regulates the α-processing of APP (Kinouchi et al., 1995; Ibarreta et al., 1999; Jolly-Tornetta & Wolf, 2000; Rossner et al., 2001; Yeon et al., 2001; Zhu et al., 2001; Kozikowski et al., 2003; Etcheberrigarray et al., 2004; Khan et al., 2009; Nelson et al., 2009) and Aβ degradation (Choi et al., 2006; Nelson & Alkon, 2009; Nelson et al., 2009). α-Processing of APP, mediated by the action of α-secretase, generates a large extracellular soluble fragment (sAPPα) and a smaller membrane-bound intracellular fragment C83. These fragments appear to exhibit no toxic properties to neurons. Evidence has been provided that the administration of bryostatin-1, a partial agonist of cPKC and nPKC isozymes, reduces Aβ40 in the brains of AD transgenic mice and both brain Aβ40 and Aβ42 in AD double-transgenic mice (Etcheberrigarray et al., 2004). Bryostatin-1 enhances at subnanomolar concentrations the secretion of α-secretase product sAPPα in fibroblasts from AD patients. In APP[V7171] transgenic mice, PKC activation has also been found to reduce Aβ40 accumulation in the brain (Etcheberrigarray et al., 2004). The action may involve an increased Aβ degradation. In APP transgenic mice, overexpression of PKCε selectively increases the activity of endothelin-converting enzyme (Choi et al., 2006), which degrades Aβ. These actions have an obvious therapeutic value to be an antidementic agent, as long as neurotoxic amyloid defines much of the pathogenesis of AD. Furthermore, PKC activation inhibits glycogen synthase 3 kinase (Lavoie et al., 1999; Fang et al., 2002) and thereby reduces tau protein hyperphosphorylation (Cho & Johnson, 2004) and intracellular neurofibrillary tangles, another main histopathological hallmark of AD. PKC activation thus reduces the pathological factors, Aβ accumulation and tau protein hyperphosphorylation, that are associated with or underlie dementia.

Third, PKC activation results in an enhancement of neurotrophic activity and synaptogenesis (see above), thus activating the endogenous neurorepairing/protective mechanisms against neurodegenerative disorders.

The combination of a memory-enhancing action, reduction in brain amyloid burden and tau protein hyperphosphorylation, and synaptic repair/synaptogenesis may represent a promising multi-target strategy with one agent and an effective therapeutic approach against AD.

6.2. Cerebral ischemia/stroke

PKC isozymes are involved in ischemic injury and tolerance, depending on isozyme types and extent of the activity. Ischemia decreases neuronal PKCε expression, a decrease blocked by hypothermia (Shimohata et al., 2007). An ischemic 'postconditioning' can induce a post-stroke increase in PKCε activity and decrease in MAPK and PKCδ activity, correlating with improved behavioral function following focal stroke (Gao et al., 2008). The PKC activator phorbol myristate has been shown to decrease brain edema following middle cerebral artery occlusion in rats (Fazzina et al., 2010). Activation of some PKC isoforms, especially the PKCε, can promote neurotrophic activity, synaptogenesis and synaptic repairing/remodeling, resulting in a rescue of ischemic impairment of spatial learning and memory (Sun et al., 2008, 2009). The results raise the possibility of post-ischemic therapy through PKC-mediated neurotrophic and repairing mechanisms.

PKC activity after ischemia not only depends on the isoforms but also on the severity of ischemia. PKC activity increases with milder ischemia (Sieber et al., 2009) and decreases after severe ischemia. PKC, especially PKCδ, is involved in synaptic dysfunction and memory impairments in patients surviving ischemic events (cerebral ischemia, cardiac arrest, etc.) (Perez-Pinzon et al., 2005). Global cerebral ischemia increases PKCδ mRNA and protein levels in the cortex and hippocampus and these levels are also increased in the compromised peri-infarct region after local cerebral ischemia (Miettinen et al., 1996; Koponen et al., 2000). The increased PKCδ expression in the penumbral area may be responsible for the delayed neuronal damage (Phan et al., 2002). Ischemia and stroke elicit release of glutamate and rapidly activate PKC through a translocation from the cytosol to the membrane fraction, resulting in neuro-damage and neurodegeneration. Hypoxia is a powerful trigger in the response. Hypoxia activates PKC, leading to phosphorylation of NMDA NR1 subunits and an enhancement of glutamate receptor activity and Ca^{2+} influx (Bickler et al., 2004). In acutely dissociated rat CA1 neurons, oxygen and glucose deprivation after removal of extracellular Ca^{2+} can still activate PKC through endogenous Ca^{2+} release (Larsen et al., 2004), suggesting that a brief period of cerebral ischemia without exposure to excitotoxicity is sufficient to activate PKC. Global cerebral ischemia triggers a diacylglycerol kinase (DGK)ξ translocation from the nucleus to perikaryal cytoplasm of the CA1 pyramidal cells as a very early phase of ischemic insult, probably resulting in a sustained increase in diacylglycerol level and PKC activity in the nucleus (Ali et al., 2004). A selective PKCδ peptide inhibitor, for example, has been found to reduce cellular injury in a rat hippocampal slice model of cerebral ischemia, when present both during the ischemic episode and for the first 3 h of reperfusion. The inhibitor decreases infarct size in vivo in rats with transient middle cerebral artery occlusion when administered at the onset, at 1 h, or at 6 h of reperfusion (Bright et al., 2004).

Much of the PKC-mediated neurotrophic and repairing activity can also be induced by a weak "pre-stroke" ischemia, i.e., ischemic preconditioning. Ischemic preconditioning is an intrinsic adaptive condition by which mild ischemic insults make cells less vulnerable to a subsequent "lethal" ischemic insult. PKC mediates the ischemic tolerance. Activation of PKCε, as a vital part of adenosine/NMDA-activated signal transduction pathway, protects cultured rat neurons and rat organotypic hippocampal slice from ischemia–reperfusion injury (Di-Capua et al., 2003; Raval et al., 2003) and cultured mouse neurons from oxygen–glucose deprivation damage, whereas selective inhibition of PKCβI enhances astrocyte cell death induced with oxygen–glucose deprivation (Wang et al., 2004). Post-ischemic activation with intermittent doses of bryostatin-1 has been found to restore rats' neurotrophic activity and synaptogenesis in the hippocampus and spatial learning and memory performance after global cerebral ischemia (Sun et al., 2008). Thus, an appropriate activation of PKC isozymes with PKC activators may represent an effective therapeutic approach, through activating ischemic preconditioning responses, against stroke/ischemia–reperfusion injury and associated memory impairment.

PKC may also mediate neuroprotective effects of estrogen. Female animals are less vulnerable to ischemia-induced neuronal damage (Alkayed et al., 1998; Zhang et al., 1998) and estrogen treatment protects the brain from experimental stroke (Yang et al., 2000; McCullough & Hurn, 2003). Transient unilateral middle cerebral artery occlusion (90 min) followed by 22.5 h reperfusion has been shown to produce smaller total infarct size in C57BL/6 female mice than in the male mice, but such difference is not observed in PKCγ knock-out mice (Hayashi et al., 2005). Injection of estrogen (i.p.) after the start of reperfusion can significantly reduce the infarct volume in males but such a protective effect is attenuated in PKCγ-knockout mice (Hayashi et al., 2005). These data suggest that estrogen has PKC-mediated neuroprotective values against cerebral ischemia, although clinical evidence for stroke prevention with hormone replacement therapy remains inconclusive in humans.

6.3. Aging

Memory function, including hippocampus-dependent memory, declines during aging in humans. The decline is associated with a decrease in the number of synapses and synaptic responses, but not the number of hippocampal neurons (Rosenzweig & Barnes, 2003). Acute bryostatin-1 treatment enhances mushroom spine formation but not the number of axonal boutons in aged rats (Hongpaisan & Alkon, 2007b). By using a herpes simplex virus-1 vector to deliver a

constitutively active PKC, Zhang et al. (2009) have showed that genetic activation of PKCβII in the hippocampal dentate granule neurons improves spatial learning in 24-month aged rats.

7. Adverse effects and toxicity of protein kinase C agents

One major concern about PKC activators, especially for those non-selective, is that PKC isoforms are involved in a variety of vital functions and neurological disorders so that a long-term PKC activation may cause wide and severe non-therapeutic reactions. For instance, all forms of PKC isoforms are sensitive to oxidative stress. It remains to be studied whether a sustained increase in the plasma levels of bryostatin-1 would further sensitize PKC isozymes to oxidants. PKC activity is significantly increased in synaptosomal samples isolated from the forebrain, midbrain, and hind brain of spontaneously hypertensive rats (Hughes-Darden et al., 2001). In spontaneous hypertensive rats, enhanced PKC activation appears to be responsible for the enhanced basal neural activity in the anterior hypothalamic area (Kubo & Hagiwara, 2005). The impact of PKC activators on cardiovascular function remains to be evaluated. Based on the data available in clinical trials, however, bryostatin-1 is well tolerated as an antitumor agent. Its adverse side effects are rare, generally mild, and reversible. The maximum tolerated dose of bryostatin-1 in humans has been found to be about 25 $\mu g/m^2$/week, given intravenously over up to 8 weeks (Jayson et al., 1995; Mutter & Wills, 2000; Clamp et al., 2003). Toxic reactions are also rare and generally mild. Myalgia is the dose-limiting toxicity in humans. Myalgia occurs at one to two days after infusion and tends to get worse with repeated administration. The symptoms are eased by exercise but return on resting. The calves, thighs and extraocular muscles are affected first but myalgia becomes more generalized as therapy continues. Myalgia affecting the muscles of hypopharynx may result in frontal headache and odynophasia. Lasting impairment of oxidative metabolism in muscle mitochondria may be responsible for myalgia (Hickman et al., 1995). Other reported adverse reactions in humans include fatigue and lethargy; they are common but generally mild. Less common adverse effects include low-grade pyrexia, nausea and anorexia. Adverse hematological toxicities in humans are not common, although thrombocytopenia and leucopenia have been reported. Mild abnormalities of liver function have also been reported. One interesting aspect about bryostatin-1 toxicity is that it may be age-dependent. Children can tolerate higher doses of the drug, with reported maximal tolerated dose of bryotaxin-1 of 44 $\mu g/m^2$ in children (Weitman et al., 1999). Myalgia and photophobia are the dose limiting toxic effects of bryostatin-1 in children.

The downside with potent PKC activators may also come from an over-activation of the α-processing of APP and/or Aβ degradation, since APP (through an interaction with β1 integrin) and Aβ (including monomers and oligomers at picomolar levels) may function physiologically in neuronal adhesion and migration (Siemes et al., 2006; Young-Pearse et al., 2007), promoting neurite outgrowth (Perez et al., 1997; Small et al., 1999; Hoareau et al., 2008; Hoe et al., 2009) and synaptic plasticity and memory (Puzzo et al., 2008), respectively. In addition, improved memory may not always be beneficial. Examples may include fear and drug abuse. Activation of PKC is involved in the formation of conditioned cues-provoked cocaine memory (Lai et al., 2008). Drug abuse-associated cues reinstate and facilitate the drug-taking behavior (Lee et al., 2006b; Yan et al., 2007). It remains to be determined whether the involvement of PKC in methamphetamine-induced long lasting astrocytic activation and behavioral sensitization (Narita et al., 2005) would jeopardize clinical usage agents as therapeutics in some cases.

8. Summary and future directions

PKC activators exhibit some pharmacological profiles that favor their potential as memory therapeutics. These activators, at appropriate doses in vivo, enhance learning and memory, induce the maintenance and repairing of the 'hardware' and 'software' in information processing and storage, and arrest some dementic progressions. These agents may be developed as memory enhancers and/or therapeutics for memory disorders such as in cerebral ischemia/stroke, AD and other dementic disorders, as well as memory deficits in aging.

PKC activators, especially those for PKCα,ε, promote neurotrophic activity and induce synaptogenesis and network repairing. These actions may greatly facilitate post-ischemic/stroke recovery in cognition and other brain functions. The important issue in using PKC agents for postischemic therapy is the necessity of targeting the right PKC isoform(s), since activation of some isoforms, especially the PKCδ, may actually increase ischemic damage and neuronal death.

The desired pharmacological profile for the treatment of AD includes a selective activation of PKC isozymes, without inducing a significant degradation of PKC isoforms, an activation of enzymes that are involved in Aβ degradation (such as insulysin, neprilysin, or endothelin-converting enzyme), and/or an inhibition of glycogen synthase kinase-3. Some substituted pyrroline compounds exhibit a dual-action on PKC isozymes and glycogen synthase kinase 3β (Zhang et al., 2006), providing valuable leads for the development of therapeutic agents acting on the multiple targets.

Bryostatins are potent activators of PKC. Their preclinical studies reveal important therapeutic potential as cognition-enhancing and anti-dementic agents, against memory impairments in AD animal models and in cerebral ischemia/stroke. PKC activators, such as bryostatin-1, are well tolerated in clinical studies. Their potential adverse impact on sub-populations remains to be evaluated in clinical trials. Much of the adverse and side effects may be further reduced through the development of PKC isozyme-specific agents, such as DCP-LA, in the future.

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