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# Forum Review

# Role of Proteolytic Activation of Protein Kinase Cδ in Oxidative Stress-Induced Apoptosis

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## ABSTRACT

**Protein kinase Cδ (PKCδ), a member of the novel PKC family, is emerging as a redox-sensitive kinase in various cell types. Oxidative stress activates the PKCδ kinase by translocation, tyrosine phosphorylation, or proteolysis. During proteolysis, caspase-3 cleaves the native PKCδ (72–74 kDa) into 41-kDa catalytically active and 38-kDa regulatory fragments to persistently activate the kinase. The proteolytic activation of PKCδ plays a key role in promoting apoptotic cell death in various cell types, including neuronal cells. Attenuation of PKCδ proteolytic activation by antioxidants suggests that the cellular redox status can influence activation of the proapoptotic kinase. PKCδ may also amplify apoptotic signaling via positive feedback activation of the caspase cascade. Thus, the dual role of PKCδ as a mediator and amplifier of apoptosis may be important in the pathogenesis of major neurodegenerative disorders, such as Parkinson's disease, Alzheimer's disease, and Huntington disease.** *Antioxid. Redox Signal.* 5, 609–620.

## INTRODUCTION

OXIDATIVE STRESS AND APOPTOSIS contribute to the degenerative processes in many neurodegenerative diseases, including Alzheimer's disease, Parkinson's disease, Huntington disease, and amyotrophic lateral sclerosis (10, 13, 18, 78, 79). Pathological markers of oxidative insult and apoptotic cell death have been identified in many human neurodegenerative disorders. Results from studies using cell culture and animal models have extended the findings from the human pathological studies by demonstrating that oxidative stress plays a causal role in apoptosis (10, 13, 18, 78, 79). In the past 5 years, understanding of the cellular mechanisms of apoptosis in the nervous system has advanced considerably, and two major apoptotic signaling pathways have been identified: (a) the mitochondrial-dependent apoptotic cascade, and (b) the death receptor (Fas)–dependent apoptotic cascade. Although these two apoptotic pathways are conceptually distinctive, they converge at the level of the effector caspases, the key mediators of apoptotic cell death. Caspase-3 activates or inactivates many cellular substrates in order to induce DNA fragmentation. However, little is understood about the critical downstream cellular target of caspase-3 activation in the CNS that leads to DNA fragmentation. Recently, we determined that protein kinase Cδ (PKCδ), a member of the novel PKC isoform family, serves as a key substrate for caspase-3 in apoptotic cell death in cell culture models of Parkinson's disease. We also demonstrated that the caspase-3-dependent proteolytic cleavage of PKCδ not only mediates apoptosis, but also amplifies the apoptotic cascade through positive feedback activation of caspase-3. The primary focus of this review is to provide an overview of PKCδ, its mode of activation, and its pathological role in oxidative stress-dependent apoptosis.

## STRUCTURAL PROPERTIES OF PKCδ

PKCδ was originally discovered by Gschwendt *et al.* in 1986 (31) and subsequently cloned from a rat brain cDNA library (30). The PKCδ gene is localized on human chromosome 3, rat

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chromosome 16, and mouse chromosome 14 (30). The recent *Science* publication entitled *Kinome* classified the PKCδ isoform in the AGC kinase family (57). Currently, 12 identified PKC isoforms are classified into three distinct subfamily groups based on their activation patterns. These subfamily categories are entitled conventional PKC (cPKC), novel PKC (nPKC), and atypical PKC (aPKC) (30). cPKCs include PKCα, $\beta_I$, $\beta_{II}$, and γ, are dependent on intracellular calcium concentrations, and are activated by diacylglycerol (DAG) or phorbol ester. Then PKCs include PKCδ, ϵ, η, θ, and μ and are also activated by DAG or phorbol ester but are calcium-independent. The last group of aPKCs includes PKCζ and λ(ι), which require neither calcium nor phospholipids for activation. The enzymes in the different subfamilies are differentially activated primarily due to differences in their molecular structures. All PKC proteins consist of the regulatory domain (N-terminus) and the catalytic domain (C-terminus). Both cPKCs and nPKCs contain cysteine-rich sequences that interact with phospholipids and phorbol ester activators, whereas aPKC enzymes lack these sequences. Furthermore, only cPKC enzymes possess the calcium-binding region (C2) in the regulatory domain and are thus calcium-dependent (30 and references therein). The amino acid sequence homologies have been determined to be 82% (βI), 85% (βII), 75% (γ), 58% (δ), 60% (ϵ), and 51% (ζ) compared with the PKCα isoform (30 and references therein).

The PKCδ structure (Fig. 1) contains a C-terminal catalytic domain with two conserved regions, an ATP-binding region (C3), a catalytically active/substrate binding region (C4), an N-terminal regulatory domain with an inhibitory pseudosubstrate sequence, and two cysteine-rich zinc-finger-like sequences (Cys1 and Cys2) in the C1 region (30 and references therein). Functional studies have revealed that the Cys2 region may play a critical role in the translocation of cytosolic PKCδ into cellular membranes following activation by phorbol esters. Five of six cysteine residues and two histidine residues interact with $Zn^{2+}$ to form a specific coordination and attract phorbol ester binding (30 and references therein).

## DISTRIBUTION OF PKCδ IN THE CNS

PKCδ is ubiquitously expressed in most tissues and cell types (50). The expression of PKCδ in different murine tissues has been evaluated, and high levels of PKCδ have been found in the epidermis, placenta, uterus, brain, lung, and kidney (50). In the CNS, PKCδ, as well as PKCα, β, ϵ and ζ, is present at birth, and the expression of PKCδ increases in the brain, but decreases in the lung, kidney, and heart as age progresses (29). This age-dependent change in specific tissues may affect the responsiveness of these tissues to certain stimuli. Merchenthaler *et al.* (1993) observed abundant expression of PKCδ in the cerebellum (61). Within the cerebellum, PKCδ is highly expressed in the Purkinje cells and is restricted to the sagittal bands of Purkinje cells in the posterior cerebellar cortex (4). A uniform pattern of expression is noted in the lower folia of the uvula and nodulus of Purkinje cells (4). Additionally, PKCδ mRNA is expressed in the thalamus, the habenula, the septum, and the cerebellar granule cells of rat brain. Using western blot and immunochemical methods, Oehrlein *et al.* (1998) showed that PKCδ along with $PKC\beta_{II}$, PKCϵ, and PKCη was up-regulated 3 days after primary hippocampal neurons were treated with retinoic acid, but decreased after day 6 when the phenotypic neuronal development was complete (66). Increased expression of PKCδ in the hippocampus and cortex was reported following kainic acid injection (37, 58). We observed a high level of PKCδ expression in the dopamine-rich brain areas, including the striatum and substantia nigra, in both rat and mouse brain (unpublished observations).

## ACTIVATION MECHANISMS OF PKCδ

PKCδ is activated by a variety of stimuli including reactive oxygen species (ROS) (43, 56), chemicals (3, 72), ultraviolet radiation (16), growth factors (22), and cytokines (14). Based on the current literature, PKCδ can be activated by any of three modes (Fig. 2): (a) membrane translocation, (b) tyrosine phosphorylation-dependent activation, and (c) caspase-3-dependent proteolytic activation.

### *Membrane translocation*

PKCδ is primarily activated by translocation to the cellular membrane during stimulation with the lipid signaling molecules phospholipid, DAG, or phorbol ester [phorbol 12-myristate 13-acetate (PMA)] (Table 1). Upon binding of the cysteine-rich domain (C1) to PMA, phospholipid, or DAG, the cat-

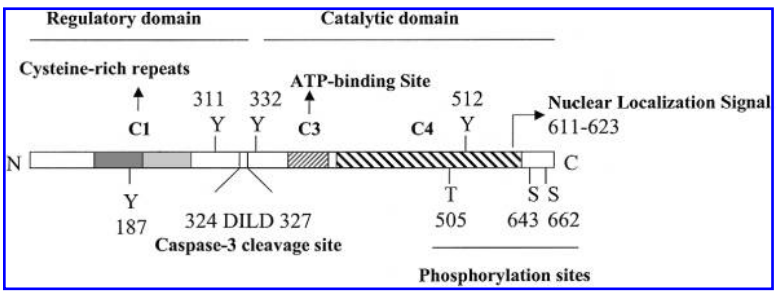


**FIG. 1. The structural and functional features of PKCδ.** The domain structure of PKCδ is schematically shown, along with the caspase-3 cleavage site, ATP-binding sites, nuclear localization signal, and phosphorylation sites of serine (S), threonine (T), and tyrosine (Y) residues.

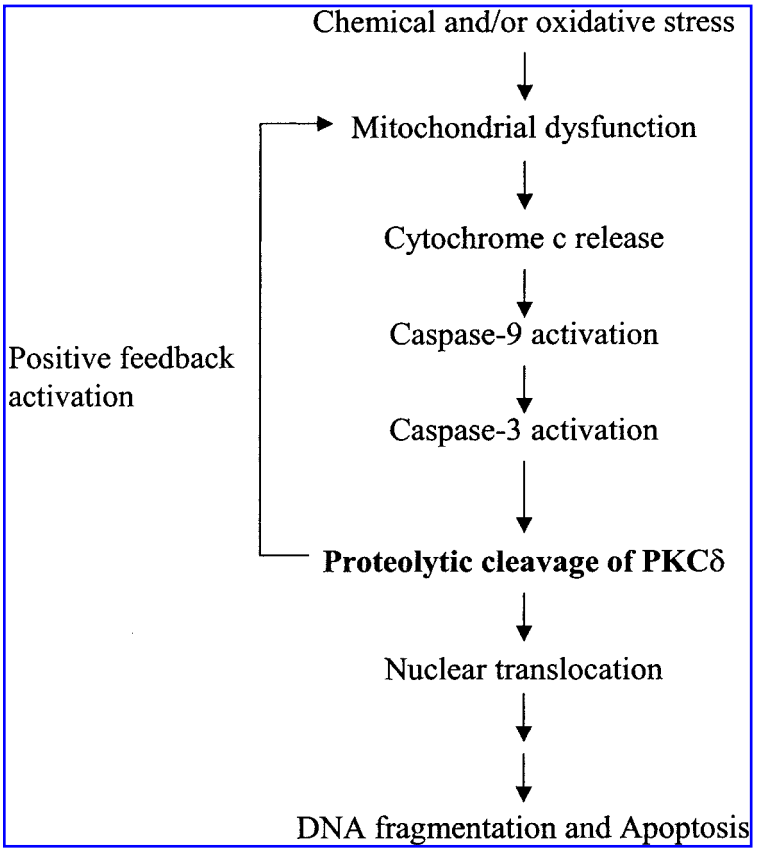


**FIG. 2. Schematic representation of the proapoptotic role of PKCδ in oxidative stress-induced apoptosis.** Exposure to environmental neurotoxins increases ROS production and disrupts mitochondrial function, which results in the release of cytochrome *c* into the cytosol. Cytosolic cytochrome *c* activates caspase-9, which then activates caspase-3. Caspase-3 then cleaves PKCδ, resulting in a persistently active catalytic fragment. Proteolytically activated PKCδ translocates to the nucleus and mediates DNA fragmentation. Catalytically active PKCδ fragment also activates caspase-3 via a possible feedback activation loop.

alytic domain is exposed, allowing substrates to bind to the site. Many conserved serine and/or threonine phosphorylation sites regulate the activity of PKC isoforms. Thr-505, Ser-643, and Ser-662 are three phosphorylation sites in PKCδ, and phosphorylation of these sites by other kinases influences the activity of PKCδ. Phosphorylation of S643 appears to be required prior to kinase activation. Mutation of S643A causes a >70% decrease in kinase activity (54). Further investigation

TABLE 1. TRANSLOCATION OF PKCδ IN RESPONSE TO APOPTOTIC STIMULI*

| *Apoptotic stimuli* | *Cell type* | *Translocation to* |
|---|---|---|
| UV radiation | Keratinocytes | Membrane |
| PMA | Keratinocytes | Mitochondria |
| PMA | U397 leukemia cells | Mitochondria |
| $H_2O_2$ | U397 leukemia cells | Mitochondria |
| UV radiation | Keratinocytes | Mitochondria |
| γ irradiation | MCF-7 | Nucleus |
| Cytosine arabinoside | HL-60 | Nucleus |
| Etoposide | C6 glioma | Nucleus |
| FAS ligation | T lymphocytes | Nucleus |
| IL-2 deprivation | T lymphocytes | Nucleus |
| Ceramide | HeLa | Golgi |
| Interferon-γ | HeLa | Golgi |
| Sindbis virus | C6 glioma | Endoplasmic reticulum |

*11, 21, 31, 39 and references therein.

revealed that S643 is autophosphorylated, thus enhancing catalytic activity, when PKCδ is in a low activity form (49). However, Stempka *et al.* (1999) reported that S643A did not reduce kinase activity (81). More must be learned to define the role of S643A. Phosphorylation of T505 in the activation loop and S662 in the hydrophobic C-terminus appears to be important for PKCδ activation because unphosphorylated T505 and S662 sites in PKCδ resulted in <1/10 of the normal kinase activity (49). A threonine residue (T505) in the activation loop of PKCδ may be phosphorylated by 3-phosphoinositide-dependent protein kinase 1 (PDK1) (32). Additional serine and threonine sites in PKCδ may facilitate regulation of kinase activation. Furthermore, PKCδ translocates to the nucleus, mitochondria, cytoplasm, plasma membrane, and other cellular organelles to initiate programmed cell death (16, 23, 56). A new class of PKC anchoring proteins called receptors for activated C-kinase (RACKs) and caveolins help target the PKC isozymes to the different organelles within the cell and facilitate the interactions between the individual PKCs and their substrates (74). RACKs maximally bind to the regulatory subunit of PKCs in the presence of lipids or $Ca^{2+}$, suggesting that they bind to activated PKCs. A number of different RACKs and their corresponding PKC partners have been identified. For example, RACK1 binds and anchors $PKC\beta_{11}$ (89); similarly RACK2 is specific for PKCε (74). RACK2 is a vesicular protein presumably involved in vesicular release and cell–cell communication. Recently, an unidentified RACK protein was reported to be associated with PKCδ (17). Isoform-specific novel peptide inhibitors or activators of PKCs have recently been developed based on the binding sequence of RACK (63) and may become useful experimental tools to study the biological roles of PKCs in the CNS. Finally, the expression RACKs is regulated in an age-dependent manner; their importance is evident from the fact that PKC translocation does not occur in the aged rat brain because RACK1 protein levels are reduced (5).

### *Tyrosine phosphorylation-dependent activation*

In addition to the serine and threonine phosphorylation sites, tyrosine phosphorylation is also important in modulating PKCδ activity (Table 2). Tyrosine 52, 155, 187, 311, 332, 512, 523, and 565 of PKCδ are phosphorylated and regulate kinase activity. In particular, tyrosine phosphorylation at positions 311, 332, and 512 induces activation of PKCδ in HaCaT and COS-7 cells following exposure to hydrogen peroxide and UV-B, respectively (39). Tyrosine phosphorylation at these positions may cause conformational changes and open the catalytic domain (39), because two major tyrosine phosphorylation sites (Y311 and Y332) are strategically located in the hinge region of PKCδ. Upon phosphorylation of these residues, PKCδ may undergo conformational change and expose the catalytic domain, yet further studies are necessary to confirm this hypothesis. Several different tyrosine kinases, including Src, Fyn, Lyn, c-Abl, protein tyrosine kinase 2 (PYK2), Lck, and growth factor receptors, are involved in phosphorylation of PKCδ and some of the phosphorylation sites seem to be isoform-specific (11, 21, 31, 39 and references therein). Regulation of kinase activity by tyrosine phosphorylation is particularly important for PKCδ because PKCδ is most efficiently tyrosine-phosphorylated among the PKC family. Tyrosine phosphorylation negatively modulates PKCδ activity in epidermal growth factor-treated epidermal cells and phosphorylation by Src family enzymes (39 and references therein). On the other hand, tyrosine phosphorylation positively modulates PKCδ activ-

TABLE 2. REGULATION OF PKCδ ACTIVITY BY PHOSPHORYLATION*

| *Stimulus* | *Sites* | *Phosphorylated by* | *Functional significance* |
|---|---|---|---|
| $H_2O_2$ | S643 | Autophosphorylated | Lipid-independent increase in PKCδ activity |
| $H_2O_2$ | S662 | PI3K | |
| $H_2O_2$ | T505 | PDK1, PI3K | Stability |
| IgE antigen, Sindbis virus | Y52 | | |
| Sindbis virus, etoposide | Y64 | | |
| PMA, Sindbis virus | Y155 | Lyn | |
| PDGF, toposide, IgE antigen | Y187 | | Facilitates translocation to the nucleus for caspase-3-dependent cleavage |
| $H_2O_2$ | Y311 | Src | Facilitates translocation to the mitochondria to mediate cyt *c* release, promotes degradation |
| $H_2O_2$ | Y332 | | Facilitates translocation to the mitochondria to mediate cyt *c* release, promotes degradation |
| $H_2O_2$, UV radiation | Y512 | c-Abl | |
| $H_2O_2$ | Y523 | | |
| $H_2O_2$ | Y565 | Lyn | |

*11, 21, 31, 39 and references therein.

ity when cells are treated with phorbol ester, nerve growth factor, and substance P. Another amino acid in the catalytic domain, Glu-500 (E500), contributes to the negative charge in the activation loop, which is critical for kinase activity. Kinase activity in E500V PKCδ was reduced by ~75% in a mutation study, however, the detailed mechanisms are still unclear at this point (39 and references therein).

### *Caspase-3-dependent proteolytic activation*

Recent studies have demonstrated a novel form of PKCδ activation in which the kinase is proteolytically cleaved in response to a variety of apoptotic stimuli in diverse cell types (11, 24, 39 and references therein). Emoto *et al.* (1995) first reported that PKCδ can be proteolytically cleaved by an ICE-like protease in response to ionizing radiation (24). Using the caspase-3 specific inhibitor Z-Asp-Glu-Val-Asp-fluoromethyl ketone (Z-DEVD-FMK), later studies established that proteolytic cleavage of PKCδ is mediated by caspase-3 (11, 23, 24, 39 and references therein). The caspase-3 cleavage site in PKCδ was identified as the V3-domain adjacent to the aspartic acid at the DMQD330N site, indicating that PKCδ is an endogenous substrate for caspase-3. Caspase-3 cleaves the kinase to yield 41-kDa catalytically active and 38-kDa regulatory PKCδ fragments to permanently dissociate the PKCδ regulatory subunit from the catalytic subunit and persistently activate the kinase. The proteolytic activation of PKCδ has mainly been implicated in the apoptotic cell death of many cellular systems. The proapoptotic activity resulting from proteolytic activation was demonstrated by the finding that overexpression of the PKCδ catalytic fragment was alone sufficient to induce cellular apoptosis, whereas apoptosis was not observed in cells overexpressing the dominant negative mutants of full-length PKCδ or the kinase-inactive catalytic fragment. Furthermore, overexpression of a mutated PKCδ (the aspartate residue in position 327 was mutated to alanine) in the caspase cleavage site protected cells from etoposide-induced apoptosis (23).

Proteolytic cleavage of PKCδ has been observed in a number of nonneuronal cell types in response to various apoptotic stimuli, including etoposide, cytosine arabinoside, γ-irradiation, mitomycin, tumor necrosis factor-α (TNFα) Fas ligation, *cis*-diamminedichloroplatinum (II) (cDDP), UV radiation, interleukin-1β (IL-1β), and streptozotocin (11, 21, 31, 39 and references therein). PKCδ cleavage was also observed during spontaneous apoptosis of neutrophils. Table 3 summarizes reports of proteolytic activation of PKCδ during apoptosis. Recently, we characterized the role of proteolytic activation of PKCδ in the neuronal pheochromocytoma (PC12) cell and rat mesencephalic dopaminergic neuronal (N27) cell models (3) following exposure to dopaminergic neurotoxic agents, such as methylcyclopentadienylmanganese (MMT) (3), dieldrin (41), and 1-methyl-4-phenylpyridinium ($MPP^+$) (unpublished observations). These chemicals dose-dependently induced the rapid generation of ROS, which subsequently triggered release of cytochrome *c* and activation of caspase-9 and caspase-3 in PC12 and N27 cells. Dopaminergic toxins also proteolytically cleaved native PKCδ into 41-kDa catalytic and 38-kDa regulatory subunits to activate the kinase. The proteolytic cleavage of PKCδ and induction of kinase activity were completely inhibited by pretreatment with a caspase-3 inhibitor Z-DEVD-FMK, indicating that the proteolytic activation of PKCδ is caspase-3-dependent. Furthermore, either inhibition of PKCδ activity or overexpression of the kinase-inactive $PKC\delta^{K376R}$ mutant almost completely attenuated the neurotoxin-induced apoptosis. The proapoptotic function of PKCδ was further confirmed by intracellular delivery of catalytically active recombinant PKCδ into PC12 and N27 cells. In addition to these dopaminergic neuronal models, PKCδ cleavage was also observed in KCl-deprived cerebellar granule cell apoptosis; however, the authors did not characterize the caspase-3 dependency of PKCδ cleavage (88).

TABLE 3. PROTEOLYTIC CLEAVAGE OF PKCδ IN RESPONSE TO APOPTOTIC STIMULI*

| *Apoptotic stimuli* | *Cell type* |
|---|---|
| Etoposide VP-16 | U937, salivary gland |
| TNFα | U937, activated human T cells |
| Aplidin | HeLa |
| Dieldrin | Rat N27 mesencephalic clonal cells, PC12 |
| MMT | Rat N27 mesencephalic clonal cells, PC12 |
| $MPP^+$ | Rat N27 mesencephalic clonal cells, PC12† |
| cDDP | HeLa |
| Mitomycin | Human gastric adenocarcinoma cells |
| γ irradiation | U937 |
| UV radiation | Keratinocytes, HaCaT cells |
| IL-1β and IL-2 | Rat INS-1 pancreatic β cell, activated human T cells |
| Streptozotocin | Rat INS-1 pancreatic β cell |
| Spontaneous | Neutrophils |
| KCl deprivation | Cerebellar granule cells |
| Ara C | U937 |

*3, 11, 21, 31, 39, 41, and references therein.
†Unpublished observations.

## PKCδ PHOSPHORYLATION

Upon activation, PKCδ phosphorylates serine/threonine residues in specific substrates, although the specific amino acid substrate sequences phosphorylated by PKCδ are not well characterized. Based on a study conducted with synthetic peptides, a general consensus phosphorylation site motif for PKC was identified as RXX(S/T)XRX, where X can be any amino acid (68). Recently, Nishikawa and his colleagues examined the specific amino acid sequence motifs of major PKC isozymes and identified the common and different amino acid substrate sequences among the PKC isozymes (65). In particular, PKCδ requires a hydrophobic amino acid at position +1 in the C-terminal of the phosphorylation site (Ser), basic amino acids at positions −6, −5, −4, and −2, and glycine at position −1. All the PKC isozymes evaluated require substrates with arginine at position −3, which reconciles with the consensus sequence motif. Their data also indicate that the optimal amino acid sequence for PKCδ phosphorylation is A(A/R)**R**(K/A)**RKG**<u>**S**</u>**F**F(Y/F)G**G**, where the underlined serine (S) is the phosphorylation site and the bold letters are particularly important for sequence recognition by PKCδ. Some PKC isozymes share

common motifs, indicating that PKC phosphorylation sites may be conserved among the isozymes (65).

## PHYSIOLOGICAL ROLE OF PKCδ

The cell-specific physiological roles of PKC isoforms appear to be diverse. PKCδ plays an important role in cell differentiation and proliferation, as well as in secretion. The physiological roles of PKCδ have been characterized using various biochemical approaches, including down-regulation, antisense knockdown, and overexpression of the native or dominant negative mutant. PMA-induced down-regulation results have shown that PKCδ is involved in the stimulation of the $NA^+/H^+$ exchanger, phosphoinositide hydrolysis, prostaglandin$^2$ formation, and keratinocyte differentiation (11, 21, 31, 39 and references therein). Dominant negative mutants and antisense analysis have revealed that PKCδ plays a role in Sis-induced transformation of NIH 3T3 cells via the PMA responsive element, differentiation of murine erythroleukemia cells, and α-adrenergic activation of Na-K cotransport (11, 39 and references therein). Overexpression of PKCδ induces growth inhibition, changes in cell morphology, decreased cell density, and G1 cyclin suppression in a number of different cell types, including human glioma, NIH 3T3 fibroblasts, CHO cells, smooth muscles, and capillary endothelial cells. The catalytic domain of PKCδ induces differentiation of murine myeloid 32 D into mature macrophages in overexpressed cells upon treatment with PMA or platelet-derived growth factor (PDGF) (11, 39 and references therein). Selective activation of PKCδ facilitates nerve growth factor-induced neurite outgrowth and differentiation in PC12 cells, as well as differentiation and growth arrest in human tumor CaCo-2 cells, (11, 21, 39 and references therein). PKCδ has been implicated in pre- and postnatal developmental phases (29), consistent with its regulatory role in cell proliferation and differentiation. Endocrine secretion is also regulated by PKCδ. Neurotensin secretion from pancreatic cells is activated upon translocation of PKCδ into the membrane (53), indicating that kinase activity may be important for secretion of certain hormones. Up-regulation of PKCδ increases L-type $Ca^{2+}$ channels in PC12 cells (28). Furthermore, PKCδ may play a role in the release of γ-aminobutyric acid in Purkinje cell axon terminals (4). Recently, two labs independently demonstrated enhanced B-cell proliferation and development of autoimmune disease in PKCδ knockout mice, establishing a critical role for PKCδ in immune modulation (60, 62). PKCδ knockout mice will assist greatly in clarifying the role of PKCδ in many pathological states. Our laboratory is currently studying the role of PKCδ in the neurodegenerative process using the knockout mice.

## REDOX MODULATION OF PKCδ ACTIVITY

Oxidative stress is implicated in apoptotic cell death in both neuronal and nonneuronal cells. Recent studies indicate that reactive oxygen radicals directly modulate PKCδ activity. Majumder *et al.* (2001) demonstrated that PKCδ translocates to mitochondria and alters mitochondrial function, including cytochrome *c* release, following exposure to hydrogen peroxide in the diverse U937, MCF-7, 293T, and NIH 3T3 cells (56). In contrast, Konishi *et al.* (1999) found that stimulation of PKCδ-overexpressing CHO cells with hydrogen peroxide results in activation of PKCδ that requires neither membrane translocation nor caspase-3-mediated proteolytic cleavage (42). They attributed the hydrogen peroxide-induced increase in PKCδ activity to tyrosine phosphorylation at Tyr-311, Tyr-332, and Tyr-512. The mutation at Tyr-311 prevented hydrogen peroxide-stimulated PKCδ enzyme activity, suggesting that phosphorylation of the Tyr-311 residue located between the regulatory and catalytic domain plays a critical role in oxidative modification of the kinase. Alternatively, hydrogen peroxide increases the association between PKCδ and the novel tyrosine kinase c-Abl, thereby promoting PKCδ translocation to mitochondria in HeLa cells (84). We found that hydrogen peroxide induces caspase-3-dependent proteolytic activation of PKCδ in rat mesencephalic dopaminergic neuronal cells within 3 h of exposure (unpublished observations). Thus, redox modification of PKCδ activity may be dependent on cell type, and additional study is needed to understand PKCδ activation during oxidative stress in different types of neurons.

## ROLE OF PKCδ IN APOPTOSIS

An apoptotic stimulus from the cell membrane is transmitted to the nucleus through a series of complex signaling molecules. Both receptor-mediated and mitochondrial-dependent apoptotic pathways are regulated by various kinases, including mitogen-activated protein (MAP) kinase, phosphatidylinositol 3-kinase (PI3K)/AKT, and PKCs. PKCs have been considered antiapoptotic molecules, but emerging studies may redefine the role of individual PKC isoforms in apoptotic regulation. Based on the available evidence, PKCα, PKCε, and PKCτ mainly function as antiapoptotic kinases, whereas PKCδ, PKCθ, and PKCμ have been associated with proapoptotic functions (11, 21, 31, 39 and references therein).

PKCδ activity is important during apoptosis induced following treatment with several apoptotic stimuli, including hydrogen peroxide, UV-B radiation, TNFα, PMA, ceramide, etoposide, cisplatin, methylglyoxal, IL-1β, and anti-Fas antibody (11, 21, 31, 39 and references therein). Three modes of PKCδ activation, i.e., translocation, proteolytic cleavage, and phosphorylation, promote apoptosis. We showed that proteolytic activation of PKCδ in neuronal cells induces apoptosis following exposure to the environmental neurotoxic agents MMT (3) and dieldrin (41). The proapoptotic function of PKCδ is well established, but the events downstream of PKCδ and those that lead to apoptosis remain unclear. Over the last few years, many signaling molecules that interact with PKCδ have been identified (Table 4). Cellular functions of some of the interacting proteins have been identified. PKCδ regulates the activity of other cell signaling molecules, such as scramblase, an enzyme that induces bidirectional movement of phospholipids across the membrane during apoptosis (26), DNA protein kinase (DNA-PK), an enzyme essential for the repair of double-stranded DNA breaks (7), small heat-shock proteins-25/27 (55), histone

TABLE 4. PKCδ INTERACTING PROTEINS

| *PKCδ interacting proteins* | | *Interaction** | *References* |
|---|---|---|---|
| β-Tubulin | Structural protein | A, P | 17 |
| 14-3-3ζ | Adapter/scaffold protein | A | 20 |
| 4E-BP1 | Eukaryotic initiation factor 4E (eIF4E) binding protein 1 | A, P | 46 |
| c-Abl | Tyrosine kinase | A, P, S | 6 |
| Caspase-3 | Cysteine protease | S | 24 |
| CPI-17 | Myosin phosphatase inhibitory protein | P | 25 |
| DF3/MUC1 | Mucin-like glycoprotein | P | 71 |
| DIK | Novel serine/threonine kinase | A | 8 |
| DNA-PK | DNA protein kinase | P | 7 |
| eEF-α | Elongation factor eEF-1α | P | 38 |
| Fyn | Tyrosine kinase | S | 22 |
| Gadd45 | Growth arrest and DNA-damage-inducible gene | A | 51 |
| Histone 2B | Nucleosomal core histones member | P | 2 |
| HnRNP-K | Heterogeneous nuclear ribonucleoprotein K | P | 75 |
| HSP-25/27 | Small heat shock proteins | P | 55 |
| JAK2 | Tyrosine kinase | A, P | 45 |
| Lamin B | Nuclear lamin protein | P | 19 |
| Lck | Tyrosine kinase nonreceptor type | S | 43 |
| Lyn | Tyrosine kinase | S | 93 |
| MDC9 | Metalloprotease-disintegrin family member (a.k.a. meltrin-gamma/ ADAM9) | A | 34 |
| MEKK1 | MAP kinase kinase | P | 93 |
| p300 | Histone acetyltransferase/transcription coactivator | P | 94 |
| P32 | Multifunctional chaperone protein | A | 83 |
| p73β | Structural and functional homologue of the p53 tumor suppressor | A, P | 70 |
| PDK1 | 3-Phosphoinositide-dependent protein kinase-1 | S | 32 |
| PI3K | Phosphatidylinositol 3-kinase | S | 73 |
| PKD | Protein kinase D | A, P | 85 |
| PLD2 | Phospholipase D2 | P | 59 |
| Pleckstrin | Major substrate of PKC | P | 12 |
| PP2A | Protein phosphatase 2A | D | 80 |
| PRK2 | PKC-related kinase 2 | P | 32 |
| PSA | Polysialyltransferase proteins | A, P | 27 |
| PTEN | Phosphotyrosine/PI-3P phosphate phosphatase | D | 67 |
| PTPα | Protein tyrosine phosphatase α | P | 82 |
| PYK2 | Tyrosine kinase | S | 91 |
| Rac | Stress-activated protein kinase | A, S | 76 |
| RACK | Receptor for activated PKC | A | 17 |
| RAFT1 | Rapamycin and FK506-binding protein target (a.k.a. FRAP/mTOR) | A | 46 |
| Scramblase | Phospholipid scramblase | P | 26 |
| SEK-1/MKK-4 | Stress-activated protein kinase | A, S | 76 |
| SHPTP1 | Src-homology protein tyrosine phosphatase 1 | A, P | 92 |
| SRBC | Serum deprivation response sdr gene product | A, P | 33 |
| Src | Tyrosine kinase | S | 9, 77 |
| STAT-1 | Signal transducer and activator of transcription-1 | P | 86 |
| STAT-3 | Signal transducer and activator of transcription-3 | A, P | 1, 76 |
| SynDecan-4 | Heparan sulfate-carrying core proteins | P | 64 |

*A: proteins colocalized or coimmunoprecipitated with PKCδ; D: PKCδ is dephosphorylated by these proteins; P: proteins phosphorylated by PKCδ; S: PKCδ either is phosphorylated or serves as a substrate for these proteins.

H2B (2), and lamin kinase (19). Additionally, PKCδ mediates phosphorylation of other signaling proteins, such as MAP kinases (16), the tyrosine kinase Jak2 (45), and Stat3 signal transducers and activators of transcription (35). Most recently, PKCδ has been shown to activate the redox-sensitive transcription factor nuclear factor-κB and thereby promote apoptosis in neutrophils (87). Together, activation of the proapoptotic kinase PKCδ influences the function of many other downstream signaling molecules, resulting in the rapid onset of apoptotic cell death.

Despite the prominent proapoptotic function of PKCδ, some studies have reported antiapoptotic effects of the kinase in various cell types. TNFα, fibroblast growth factor, serum deprivation, nitric oxide, and SVN1 viral stimulation exert an anti-

apoptotic effect through activation of PKCδ in human neutrophils (40), granulosa cells (69), PC12 cells (90), macrophages (36), and glioma cells (95), respectively.

## FEEDBACK ACTIVATION OF THE CASPASE-3 CASCADE BY PKCδ

Recent studies from our laboratory and others indicate that proteolytic activation of PKCδ not only mediates, but may also regulate upstream caspase-3 via a positive feedback amplification loop (3, 52, 72). The feedback regulation of caspase-3 is mainly supported by two observations: (a) inhibition of caspase-3 activity by a PKCδ inhibitor, and (b) decreased caspase-3 activity in a dominant negative PKCδ mutant. Additionally, we showed that intracellular delivery of catalytically active recombinant PKCδ alone enhanced caspase-3 activity in PC12 cells and mesencephalic dopaminergic neuronal cells. We also demonstrated that recombinant PKCδ induces cytochrome *c* release and caspase-9 activation (unpublished observation). Very recently, Leverrier *et al.* demonstrated activation of capase-3 in pituitary adenoma cells transfected with the catalytic domain of PKCδ (52). Interestingly, they also showed that expression of the PKCε catalytic domain induces proteolysis of PKCδ, suggesting that PKCε is upstream of the feedback regulation of caspase-3 (52). Nevertheless, the existence of a feedback loop provides a unique role for PKCδ in the amplification of neuronal apoptosis.

## PATHOLOGICAL ROLE OF PKCδ IN THE CNS

PKC isozymes have been implicated in many diseases, including carcinogenesis, pulmonary disorders, cardiac ischemia, drug-induced cell injury, and behavioral abnormalities. The pathological role of PKCδ in CNS abnormalities is not well established because the proapoptotic function of the kinase in the nervous system has just been recognized. Recently, Mochly-Rosen's group demonstrated opposing roles of PKCε and PKCδ in cardiac ischemia using an isozyme-specific activator peptide or inhibitory peptides of PKC-RACKs (63). They showed that the PKCε-selective inhibitory peptide prevents the protective effect of ischemic preconditioning in neonatal cardiac myocytes (63), suggesting PKCε contributes to the protective effect. Alternatively, they demonstrated, using the PKCδ-selective agonist and antagonist, that PKCδ mediates damage induced by ischemia (15, 63). Results from studies with transgenic animals support conclusions from the peptide inhibitor studies. The opposing effects of PKCδ and PKCε have recently been demonstrated in the CNS (15). Both PKCδ and PKCα translocate to the membrane after brief global brain ischemia in the rat hippocampus, suggesting that activation of PKCα and PKCδ may be associated with ischemic preconditioning-induced tolerance (48). Table 5 summarizes the pathological role of PKCδ in both *in vitro* and *in vivo* CNS models.

PKCδ mRNA expression was up-regulated 13-fold in the cortex and the CA1 and CA3 regions of the hippocampus 1 day after kainate administration (37, 58). Strong PKCδ immunoreactivity was observed in cortical and CA1–3 pyramidal neurons on days 1 and 2, and PKCδ expression was extended up to 4 days in microglial cells after kainic acid injection. Induction of PKCδ in both neurons and microglia may be important in excitotoxic neuronal injury (37). Also, PKCδ activity was decreased in Purkinje cell terminals in animals administered labyrinthectomies (4). Consistent with our findings in cell culture models, we recently observed increased proteolytic cleavage in midbrain slices during neurotoxic chemical exposures (unpublished observation).

## CONCLUSION

In conclusion, PKCδ is a proapoptotic kinase activated by multiple mechanisms, including translocation, proteolysis, and tyrosine phosphorylation. The proteolytic activation of PKCδ is important not only in activating the downstream apoptotic cascade, but also in amplifying upstream caspase signaling (Fig. 2). PKCδ is highly sensitive to redox modulation and is activated directly by ROS. The importance of the proapoptotic function of PKCδ is emerging in oxidative stress-mediated neuronal apoptosis, and further understanding of the role of this kinase in the CNS may provide insights into the mechanisms of many acute (ischemia, stroke, traumatic brain injury) and

TABLE 5. PROAPOPTOTIC ROLE OF PKCδ IN CNS MODELS

| *Stimuli* | *Neuronal cell type* | *Mode of activation* | *Biological effect* | *References* |
|---|---|---|---|---|
| Kainate-induced excitotoxic lesion | Rat hippocampus, cortex, microglia | Membrane translocation | Apoptosis | 37 |
| Global cerebral ischemia | Rat hippocampus | Membrane translocation | IPC-induced tolerance | 47 |
| Global brain ischemia | Rat hippocampus, cortex, microglia | Prolonged induction of both mRNA and protein | Apoptosis | 44 |
| Dieldrin | Rat midbrain, N27, PC12 | Proteolytic cleavage | Apoptosis | 41 |
| MMT | Rat midbrain, N27, PC12 | Proteolytic cleavage | Apoptosis | 3 |
| $MPP^+$ | Rat midbrain, N27, PC12 | Proteolytic cleavage | Apoptosis | UNP* |
| KCl deprivation | Cerebellar granule cells | Proteolytic cleavage | Apoptosis | 88 |

*UNP: unpublished observation.

chronic (Parkinson's disease, Alzheimer's disease, Huntington disease) neurodegenerative conditions.

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## ABBREVIATIONS

aPKC, atypical protein kinase C; cDDP, *cis*-diammine-dichloroplatinum (II); cPKC, conventional protein kinase C; Cys, cysteine-rich motif; DAG, diacylglycerol; DNA-PK, DNA protein kinase; $H_2O_2$, hydrogen peroxide; ICE, interleukin-1β-converting enzyme; IL, interleukin; MAP, mitogen-activated protein; MMT, methylcyclopentadienyl manganese; $MPP^+$, 1-methyl-4-phenylpyridinium; nPKC, novel protein kinase C; PDGF, platelet-derived growth factor; PDK1, 3-phosphoinositide-dependent protein kinase 1; PI3K, phosphatidylinositol 3-kinase; PKC, protein kinase C; PKCδ, protein kinase Cδ; PMA, phorbol 12-myristate 13-acetate; PYK2, protein tyrosine kinase 2; RACK, receptor for activated C-kinase; ROS, reactive oxygen species; SVN1, a virulent strain of Sindbis virus; TNFα, tumor necrosis factor α; UV-B, ultraviolet radiation B; Z-DEVD-FMK, Z-Asp-Glu-Val-Asp-fluoromethyl ketone.

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