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Current Neuropharmacology, **2014**, *12*, **213-218**

Protein Kinase C-Related Kinase (PKN/PRK). Potential Key-Role for PKN1 in Protection of Hypoxic Neurons

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Abstract: Serine/threonine protein kinase C-related kinase (PKN/PRK) is a family of three isoenzymes (PKN1, PKN2, PKN3), which are widely distributed in eukaryotic organisms and share the same overall domain structure. The N-terminal region encompasses a conserved repeated domain, termed HR1a-c as well as a HR2/C2 domain. The serine/threonine kinase domain is found in the C-terminal region of the protein and shows high sequence homology to other members of the PKC superfamily.

In neurons, PKN1 is the most abundant isoform and has been implicated in a variety of functions including cytoskeletal organization and neuronal differentiation and its deregulation may contribute to neuropathological processes such as amyotrophic lateral sclerosis and Alzheimer's disease. We have recently identified a candidate role of PKN1 in the regulation of neuroprotective processes during hypoxic stress. Our key findings were that: 1) the activity of PKN1 was significantly increased by hypoxia (1% O_2) and neurotrophins (nerve growth factor and purine nucleosides); 2) Neuronal cells, deficient of PKN1 showed a decrease of cell viability and neurite formation along with a disturbance of the F-actin-associated cytoskeleton; 3) Purine nucleoside-mediated neuroprotection during hypoxia was severely hampered in PKN1 deficient neuronal cells, altogether suggesting a potentially critical role of PKN1 in neuroprotective processes.

This review gives an up-to-date overview of the PKN family with a special focus on the neuroprotective role of PKN1 in hypoxia.

Keywords: Hypoxia, neuroprotection, PKN, PRK, protein kinase C-related kinase, purine nucleosides, review.

INTRODUCTION

Protein kinase C-related kinase 1 (PKN1/PRK1) belongs to the protein kinase C (PKC)-related kinase family (PKN/PRK), a recently discovered subfamily of the AGC serine/threonine protein kinases [1-3]. PKNs are widely distributed in eukaryotic organisms, such as starfish, amphibians, insects and mammals [4, 5]. To date three different PKN isoforms have been described: PKN1 (PKN alpha/PRK1/PAK-1), PKN2 (PKN gamma/PRK2/PAK-2) and PKN3 (PKN beta/PRK3) [1, 6, 7]. Although PKN isoforms are intimately related they may not substitute for one another, consistent with the isoform-specific effects and varying tissue expression levels, as reviewed: [8]. The expression of PKN1 in human and rat tissue [6, 9, 10] and PKN2 in mouse tissue [11] is rather ubiquitous. However, PKN3 expression is more restricted to specific tissues including skeletal muscle, heart, liver [12] and human cancer cell lines [7]. In neurons PKN1 is the most abundant isoform and has been implicated in a variety of functions including cytoskeletal organization and neuronal differentiation [5, 13-17]. A role of PKN1 in amyotrophic lateral sclerosis (ALS) [18, 19] and in Alzheimer's disease [20, 21] has been implicated. Furthermore our own data suggested that PKN1 is a key-signaling element in purine nucleoside- and nerve growth factor (NGF)-mediated protection of hypoxic neurons [22, 23].

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BASIC STRUCTURE OF PKNs

The PKN family members PKN1, PKN2, and PKN3 share the same overall domain structure. The N-terminal region encompasses a conserved repeated domain, termed HR1a-c (for homology repeat, also known as ACC1-3) as well as a HR2/C2 domain. The latter one is related to the calcium-dependent membrane-targeting domain in PKC and contains a C-terminal auto-inhibitory region (IR) [24]. The serine/threonine kinase domain is found in the C-terminal region of the protein and shows high sequence homology to other members of the PKC superfamily [4, 5] (Fig. **1**).

REGULATION OF PKNs

The N-terminal region plays an important role in the regulation of PKNs, for reviews see: [8, 25, 26]. The HR1 region is involved in the interaction with the small GTPases Rho and Rac [2, 4, 5, 25, 27-36]. Rho, binds to PKN and induces a conformational change that allows binding to phosphoinositide-dependent protein kinase 1(PDK1), which phosphorylates PKN in the activation loop and stimulates its protein kinase activity [5, 25, 37]. The HR2/C2-like domain, does not bind Ca^{2+} as expected, but is potentially involved in the activation of PKNs by lipids or the targeting of PKNs to the membrane [4, 5, 25, 38]. The C-terminal part of the C2-

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Fig. (1). Structure of protein kinase C-related kinase (PKN).

like region functions as an arachidonic acid-sensitive auto-inhibitory region (IR) [1, 24, 25, 39-42]. N-terminally truncated PKNs e.g. by caspase cleavage [18] apparently behave as constitutively-active isoforms [1, 6, 9, 25, 43-46]. In addition, PKNs were shown to be activated by various fatty acids and phospholipids *in vitro*, although the *in vivo* significance is as yet not fully characterized too [1, 6, 7, 9, 43, 44, 47, 48].

CELLULAR PKN UPSTREAM SIGNALS

The individual PKN isoforms have been linked to selective upstream signals [8] and signaling modules like neurotrophins [22, 23] and androgen receptors [49, 50] for PKN1, Platelet-derived growth factor (PDGF) and cell surface molecule CD44 for PKN2 [51] and insulin for PKN3 [52], suggesting that each isoform is associated with different adaptor proteins [11, 53, 54]. PKNs are implicated in signal transduction as effectors of Rho, Rac, PI3K (phosphoinositide 3-kinase) and Rho-like Rho-kinase [51, 52, 55-57] and all three PKN isoforms can support Rho-dependent cell migration [8].

GENERAL FUNCTION OF PKNs

As diverse as the distribution of the PKN family are its functions, which were recently reviewed [26], including regulation of cell cycle [58], receptor trafficking [59], vesicle transport [60] and apoptosis [61]. More than 20 proteins and several peptides were shown to be phosphorylated by PKN1 and PKN2, including the cytoskeletal proteins α-actinin and vimentin, as reviewed [26]. Recently, the same authors also showed that CLIP-170 (cytoplasmic linker protein of 170 kDa) and EGFR (epidermal growth factor receptor) are substrates for PKN1 and PKN3 [26]. Data by us [16] and others [60-63] link PKN1 to several stress induced pathways.

PKN2 is involved in actin cytoskeletal organization [31], mainly through activation by Rho GTPases [5]. PKN2 also plays a role alongside Fyn in controlling cell–cell adhesion in keratinocytes [64] and the maturation of apical junctions [38]. In addition, PKN2 can modulate migration in astrocytes by up-regulating cortactin phosphorylation [51] PKN3 has been identified as an effector required for malignant cell growth, downstream of activated phosphoinositide 3-kinase (PI3K) [52]. More recently, it has been shown that knockdown of PKN3 can decrease the growth of prostate and pancreatic tumors, and prevent lung metastases in mouse models [65, 66].

ROLE OF PKN1 IN NEURODEGENERATIVE DISEASES

In neurons, PKN1 is the most abundant isoform and has been implicated in a variety of functions including cytoskeletal organization and neuronal differentiation [5, 13, 17]. PKN1 was shown to phosphorylate neurofilaments at sites important for neurofilament assembly [14, 15]. Dysfunction of neurofilament metabolism was strongly implicated in amyotrophic lateral sclerosis (ALS) and in some forms of Charcot-Marie-Tooth disease [18, 19]. In ALS, accumulating neurofilaments represent one of the earliest pathological changes seen in several transgenic mouse models of ALS [67-69]. Along this line, it was shown that caspase-mediated processing of PKN1, induced by excitotoxic glutamate release and other disease-associated insults leads to deregulation of PKN1 [18] and subsequently to a disruption of neurofilament organization, axonal transport mechanisms [18, 46] and potentially also to apoptosis [45]. Other results [20, 21], suggested a specific role for PKN in neurofibrillary tangle formation and neurodegeneration in damaged neurons in Alzheimer's disease. Authors showed that PKN phosphorylated tau protein, potentially playing an important role in the aggregation of tau into helical filaments. However, any clear evidence for the involvement of PKN1 in the pathogenesis of neurodegenerative diseases is as yet missing.

ROLE OF PKN1 IN HYPOXIC NEURONS

Hypoxic stress (1% O_2) induces an increase in cell death of PC12 neuronal cells and primary neurons [23, 70, 71]. Targeting apoptotic processes after ischemic stroke has been a key focus of neuroprotective therapeutic interventions. Numerous authors, (see reviews [72-74]) have proposed adenosine and its receptors as targets for therapeutic approaches in stroke and related disorders. We have previously studied neuronal signaling in hypoxia and observed a protective capacity of the purine nucleosides adenosine, guanosine and inosine in both PC12 cells [22, 71, 75, 76] and in primary cerebellar granule neurons [71, 77-79]; see also our latest review: [70], which was inhibited by adenosine receptor (ADORA) antagonists, as reviewed [70].

Furthermore exposure of neuronal cells to low oxygen lead to increased phosphorylation of PKN1, which was augmented by NGF and purine nucleosides [16, 22]. siRNA-mediated knockdown of PKN1 in neuronal PC12 cells leads to an increase of cell death and inhibition of neurite formation accompanied by disturbance of the F-actin-associated cytoskeleton [16]. These results complement a previous report [80], showing the binding of PKN1 to the actin bundling protein alpha-actinin in a phosphatidylinositol-4,5-bisphosphate dependent manner.

These results indicate that PKN1 may act as a key-signaling element for purine nucleoside- and potentially ADORA-associated protective mechanisms in hypoxic neuronal cells (Fig. **2**).

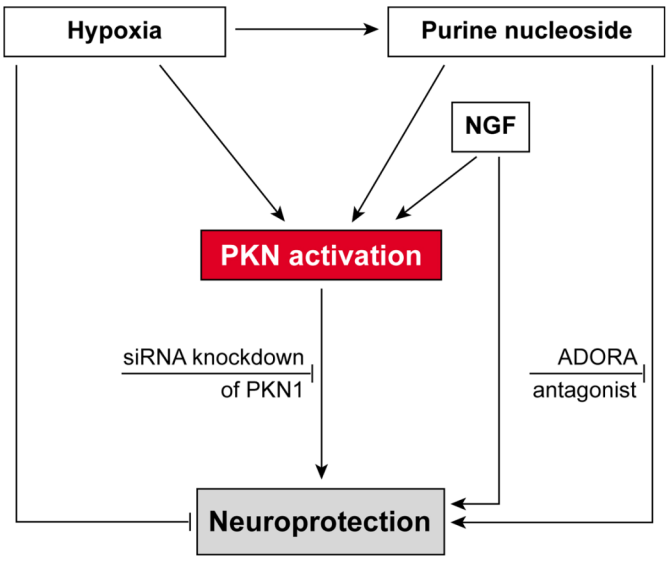

Fig. (2). Key-role of PKN1 in neurotrophin-mediated rescue of hypoxic neuronal cells.

CONCLUSION

In neurons, PKN1 is the most abundant isoform and has been implicated in a variety of functions including cytoskeletal organization and neuronal differentiation [5, 13, 17]. PKNs are also involved in regulation of stress response of neuronal cells as well as primary neurons and deregulation of PKN1 may contribute to neuropathological processes such as amyotrophic lateral sclerosis [18] and Alzheimer's disease [20, 21].

Our lab has focused on neuronal stress response during hypoxia. We observed that addition of the neurotrophins NGF and purine nucleosides (PN) resulted in significant neuroprotection [16, 71], whereby the effect associated with PN was inhibited by adenosine receptor (ADORA) antagonists, as reviewed [70]. As observed in our experiments purine nucleosides as well as NGF also lead to the activation of PKN1 and to stabilization of cell viability and neurite formation, whereas knockdown of PKN1 leads to the inhibition of PKN1 and to neurodegeneration [16, 22]. Thus, PKN1 is apparently part of a key-signaling module fostering the response to hypoxic stress and likely indispensable for neurotrophin-mediated protection of hypoxic neuronal cells [16, 22, 70].

CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

ACKNOWLEDGEMENTS

This work was supported by the Austrian Science Fund (FWF): T 421-B18, P 19578-B05 and P 26002-B24. We are grateful to Dr. C. Bandtlow and Dr. G. Baier for helpful discussions.

ABBREVIATIONS

ADORA	=	adenosine receptors
ALS	=	amyotrophic lateral sclerosis
HR	=	homology repeat
NGF	=	nerve growth factor
PI3K	=	phosphoinositide 3-kinase
PKC	=	protein kinase C
PKN1/PRK1	=	Protein kinase C-related kinase 1

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Received: July 11, 2013 Revised: August 20, 2013 Accepted: December 10, 2013