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RESEARCH ARTICLE

Internalization and signal transduction of $PrP_{106-126}$ in neuronal cells

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Abstract

Invasion of the nervous system and neuronal spread of infection are critical, but poorly understood steps in the pathogenesis of prion diseases. We have thus analyzed the internalization and signal transduction of the neurotoxic fragment of the prion protein $PrP_{106-126}$ in the rat neuroblastoma cell line B104 by fluorescence microscopy and quantification by ELISA and in primary neuronal cells from mice. Phospholipase D (PLD) is known to be an enzyme involved in the regulation of secretion, endocytosis and receptor signalling. We determined the PLD activity using a transphosphatidylation assay and could show that PLD is involved in $PrP_{106-126}$ internalization. The determination of receptor activity via quantification of ERK1/2 phosphorylation and cAMP level measurement verified the $PrP_{106-126}$-induced signal transduction in B104 cells and primary neuronal cells. $PrP_{106-126}$-induced a decrease in cAMP level in neuronal cells. These studies indicate the involvement of PLD in $PrP_{106-126}$-endocytosis and mediated cellular signalling by an unidentified inhibitory G-protein-coupled receptor and may allow the development of therapeutic agents interfering with prion uptake and/or PLD function using PLD as a possible pharmaceutical target.

Abbreviations: GPCR, G-protein-coupled receptor; PLD, phospholipase D; PA, phosphatidic acid; PC, phosphatidylcholine; PtdEtOH, phosphatidylethanol; PCR, polymerase chain reaction; ERK, extracellular signal-regulated kinases; DMEM, Dulbecco's modified Eagle's medium; FCS, fetal calf serum; SFM, serum-free medium; 2,3-DPG, 2,3-diphospho-D-glyceric acid; PMA, phorbol 12-myristate 13-acetate; PTX, pertussis toxin; ABTS, 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid).

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Introduction

Prion diseases are a group of neurodegenerative disorders characterized by spongiform changes of the neuropil, gliosis and neuronal loss (Prusiner, 1998). These changes are associated with the deposits of imperfectly folded proteinase K-resistant isoforms (termed PrP(res)) of the prion protein (PrP) in the brain. Cytokines and chemokines released by PrP(res)-activated glial cells may contribute directly or indirectly to development of the disease by enhancement and generalisation of the gliosis and via neuronal cytotoxicity (Eikelenboom et al., 2002; Burwinkel et al., 2004). Since the synthetic prion peptide $PrP_{106–126}$ shares many properties with PrP it is widely used for *in vitro* studies. Previous observations have shown that the peptide fragment of the prion protein $PrP_{106–126,}$ which is reported to account for the neurodegeneration in prion disease (Forloni et al., 1993), is internalized by neurons (McHattie et al., 1999). However, the underlying pathogenic mechanisms are still poorly understood. To date, little is known about which receptor mediates prion protein internalization and regulation of $PrP_{106–126}$-induced signal transduction in neuronal cells. For glial cells, we could show the involvement of the phospholipid-specific diesterase phospholipase D (PLD; McDermott et al., 2004) in the internalization of $PrP_{106–126}$ (Brandenburg et al., 2007).

PLD hydrolyzes phosphatidylcholine (PC) to phosphatidic acid (PA) and choline and is assumed to play an important role in signal transduction, membrane trafficking and reorganization of the cytoskeleton (Liscovitch et al., 1999). Two isoforms of PLD (PLD1 und PLD2) have been cloned in mammalian cells (Hammond et al., 1995; Frohman et al., 1999). In the nervous system, PLD isoforms are present in neurons independent of their transmitter (Klein, 2005). Interestingly, an increase in PLD1 expression and activity has been shown in the brain of scrapie-infected mice (Jin et al., 2005). In the present study, we investigated whether $PrP_{106–126}$ influences the activity of PLD and which enzyme is involved in the cellular uptake/endocytosis of $PrP_{106–126}$ in the rat neuroblastoma cell line B104 and murine primary neuronal cells. Furthermore, we tested $PrP_{106–126}$-induced signal transduction in these cells-on the basis of the determination of ERK1/2 phosphorylation and cAMP level measurement. We here report that $PrP_{106–126}$ signal transduction is mediated by an unidentified inhibitory G-protein-coupled receptor (GPCR) in neuronal cells. We provide clear evidence that PLD activity is essential for $PrP_{106–126}$-endocytosis in B104 cells.

Experimental procedure

Reagents

Human $PrP_{106–126}$ and the scrambled control peptide of $PrP_{106–126}$ were purchased from Dr. P. Henklein (Charité, Berlin, Germany). Peptides were dissolved at 10 mM concentration in distilled water and show a fibrillar form. Aggregates were verified by electron microscopy (data not shown). 2,3-DPG, pertussis toxin (PTX), phorbol 12-myristate 13-acetate (PMA), and forskolin were purchased from Sigma-Aldrich (Germany).

Cell Culture

Rat neuroblastoma cell line B104 was subcultivated in Dulbecco's modified Eagle's medium (DMEM) supplemented by 10% fetal calf serum (FCS). The catalytically inactive (K758R) PLD2 mutant (nPLD2; Koch et al., 2005) was transfected in B104 cells using Lipofectamine 2000 (Invitrogen) according to the manufacturer's protocol and selected in the presence of 500 μg/ml G418 (Sigma, Germany).

For primary neuronal cell cultures, tissues were taken from Balb/c mice embryos at gestational day 15/16 and cultured as described previously (Braun et al. 2009). Briefly, pregnant females were anesthetized with CO_2 vapor, killed by cervical dislocation, and subjected to caesarean section. Cerebral cortices were dissected from fetuses. Tissues were minced, then the pieces were digested with trypsin (0.1% for 15 min at room temperature, Sigma, Germany), triturated in the presence of 10% fetal bovine serum (Gibco, Germany) and DNAse I (170 U/ml, Sigma, Germany), and finally centrifuged for 5 min at 100*g*. Cell pellets were suspended in neurobasal medium (Gibco, Germany) supplemented with B27 (0.2%, Gibco, Germany) and plated at a density of 1.5×10^5 cells/cm^2 onto poly-ornithine (Sigma, Germany)-coated well plates (Greiner, Germany). This procedure typically yields cultures that contain $>$90% neurons and $<$10% supporting cells. Cultures were maintained at 37 °C in a humidified atmosphere containing 5% CO_2 for 8 days prior to viability analysis.

RNA isolation and RT-PCR

Cells were seeded into 9.6-cm^2 culture dishes. Total RNA was isolated using the Trizol reagent (Gibco BRL, Life Technologies, USA), according to the manufacturer's recommended protocol. RNA