Prion channel proteins and their role in vacuolation and neurodegenerative diseases

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Abstract The prion encephalopathies, which are characterized by neuropathological changes that include vacuolation, astrocysis, the development of amylod plaques and neuronal loss, are associated with the conversion of a normal cellular isoform of prion protein (PrPc) to an abnormal pathologic scrapie isoform (PrPSc). The use of PrP[106–126] and its isoforms in studies of channels in lipid bilayers has revealed that it forms heterogeneous channels reflecting modifications in the peptide’s structure and differences in the properties of the formed oligomeric aggregates and their intermediates. We propose that the accumulation of pathologival isoforms of prion are linked to membrane abnormalities and vacuolation in prion diseases. The interlinked changes in membrane fluidity and endogenous channels induced by prion isoforms can occur independently and concurrently with channel formation, i.e. they are not mutually exclusive. We suggest that vacuolation is a cellular response triggered in order to immobilize pathological prion isoforms having the ability to form channels that compromise cellular membranes. This mechanism is similar to that of other channel-forming proteins that induce vacuolation, e.g. the well-established VacA of Helicobacter pylori, Vero cells and aerolysin, as well as melittin-induced micellization and membrane fusion. We conclude that channel formation is part of the molecular mechanisms responsible for the vacuolation associated with prion diseases. The initial vacuolation could be an adaptive cellular response to compartmentalize the increase in pathogenic prion isoforms, while an excessive accumulation of pathologic prion isoforms in later stages represents the inability of the cell to continue to compartmentalize these misfolded proteins in vacuoles.

Keywords Prions - Vacuolation - Channel-forming peptides

Introduction

Prion-related encephalopathies are associated with the conversion of a normal cellular isoform of prion protein (PrPc) to an abnormal scrapie isoform (PrPSc). This plays a crucial role in the physiological mechanisms underlying many neurodegenerative diseases in humans and animals, such as kuru, Creutzfeldt-Jakob disease (CJD), scrapie and bovine spongiform encephalopathy (BSE) or “mad cow” disease (Mobashery et al. 1997). The conversion of the single polypeptide prion chain to the PrPSc isoform involves a decrease in the amount of α-helix structure and an increase in β-sheet content. This change in the content ratio of α-helices to β-sheets may explain the diversity in the proposed mechanisms of action. The precise molecular steps in the above mechanisms that lead to vacuolation (Fig. 1) and the formation of spongiform lesions in transmissible encephalopathies are not well known (Armstrong et al. 2000; Foster et al. 2001). Laszlo et al. (1992) reported that in scrapie-infected brain, lysosomes and lysosome-related structures (multivesicular and tubulovesicular dense bodies) are present in abnormally high numbers in neuronal cell processes. They hypothesized that repeated rounds of phagocytosis, lysosomal biogenesis of PrPSc, lysosomal membrane rupture, hydrolytic enzyme release and neuronal lysis lead to cell damage and cell death.

Several non-exclusive modes of action have been proposed to explain prion-induced neurodegenerative diseases. The proposed modes of action include: (1) the membrane microviscosity; (2) the intracellular Ca²⁺ homeostasis; (3) superoxide dismutase and Cu²⁺
membrane and IP₃-modulated Ca²⁺ channels in the internal membranes; and/or (2) formation of cation channels by PrP itself. These two mechanisms of action lead to changes in Ca²⁺ homeostasis that further augment the abnormal electrical activity and the distortion of signal transduction, causing cell death. The hypothesis of the interaction of PrP[106–126] with membranes and the formation of redox-sensitive and pH-modulated heterogeneous ion channels is consistent with: (1) PrP-induced changes in membrane fluidity and viscosity; (2) PrP-induced changes in Ca²⁺ homeostasis (which does not exclude changes in endogenous Ca²⁺ transport pathways and Cu²⁺ homeostasis); (3) the role of PrP as an antioxidant; and (4) the structural properties of PrP, i.e. β-sheets, protein aggregation, hydrophobicity, the functional significance of specific amino acids (e.g., methionine, histidine) and regulation with low pH (Kourie 2001).

The molecular mechanism that underlies prion pathologies involves conformational conversion from the mainly α-helix PrPc isoform to the predominately β-sheet PrPSc isoform. This conformational change suggests that the β-sheet region plays an important part in the PrPSc cytotoxicity. Also, most of the pathogenic characteristics of PrPSc have been identified in a peptide corresponding to residues 106–126 of PrP (PrP[106–126]) (Prusiner 1996). This fragment coincides with the proposed β-sheets for the prion peptide (De Gioia et al. 1994; Florio et al. 1996, 1998). De Gioia et al. (1994) and Florio et al. (1996, 1998) found that PrP[106–126] is a contributor to the physicochemical and pathogenic properties of prion peptide PrPSc, which explains the use of PrP[106–126] as a tool to characterize the pharmacological and biophysical properties of PrPSc. It is thought that PrPSc-induced cell death could be mediated via changes in Ca²⁺ homeostasis (Florio et al. 1996, 1998; Lin et al. 1997; Kawahara et al. 2000). However, it is not known how this effect is brought about. The effects of PrPSc could arise from interactions with intrinsic ion transport pathways and/or from the formation of new transport pathways; either or both would lead to abnormal electrical activity and to distortion of signal transduction, causing loss of membrane compartmentalization.

**PrP[106–126] channels**

It has been reported that the effect of PrP on Ca²⁺ homeostasis is due to PrP-induced changes in voltage-sensitive calcium channels (VSCC) (Florio et al. 1996, 1998). On the other hand, Lin et al. (1997) proposed that changes in the Ca²⁺ homeostasis can be mediated via a PrP[106–126]-formed cation channel. The PrP[106–126]-formed ion channel reported by Lin et al. (1997) is voltage independent with a conductance of 20–60 pS, as well as higher states in 100 mM NaCl, and a cation/anion selectivity ratio, e.g. $P_{\text{Na}}/P_{\text{Cl}}$, of ~2.5. However, the presented data suggest that the current flow is