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ARTICLE

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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, astrocytosis, the development of amyloid plaques and neuronal loss, are associated with the conversion of a normal cellular isoform of prion protein (PrP^{c}) to an abnormal pathologic scrapie isoform (PrP^{Sc}). 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 pathological 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

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Introduction

Prion-related encephalopathies are associated with the conversion of a normal cellular isoform of prion protein (PrP^{c}) to an abnormal scrapie isoform (PrP^{Sc}). 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 PrP^{Sc} 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 PrP^{Sc}, 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 Ca^{2+} homeostasis; (3) superoxide dismutase and Cu^{2+}

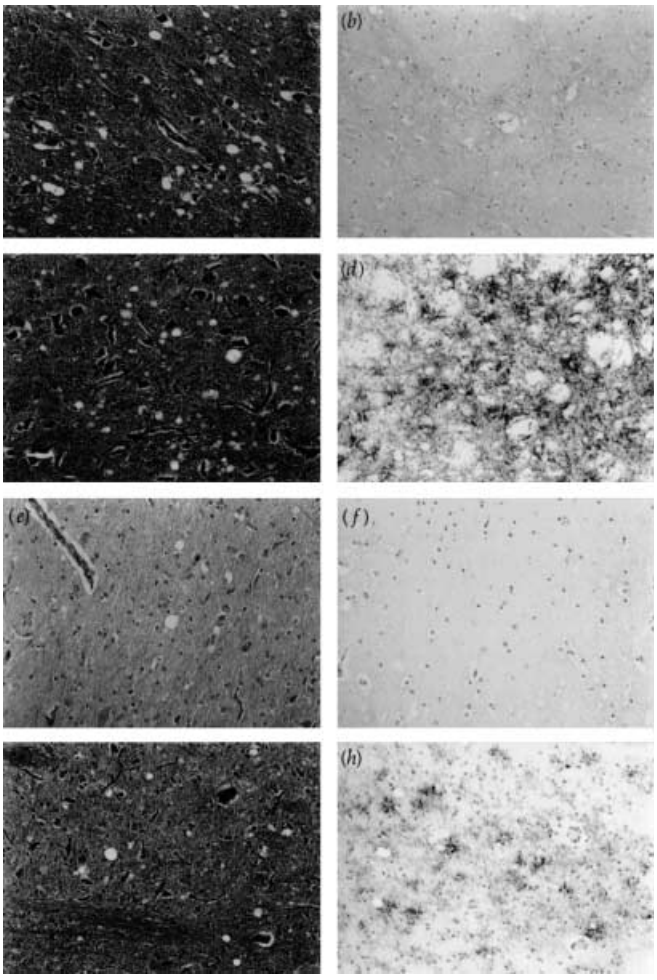

Fig. 1. **a** Haematoxylin- and eosin-stained section showing extensive vacuolation (score 5) in thalamic nuclei from goat 47×00 with terminal scrapie 659 days following intracerebral inoculation with SSBP/1 scrapie (×200). **b** Immuonhistochemical staining for PrP with BG4 antibody in thalamic nuclei of goat 47×00 showing marginal PrP staining (score <1) (×200). **c** Haematoxylin- and eosin-stained section showing extensive vacuolation (score 4) in thalamic nuclei of goat 45×45 with terminal disease 547 days following inoculation with BSE (×200). **d** Immunohistochemical staining with BG4 for PrP in thalamic nuclei of goat 45×45with terminal disease 547 days following inoculation with BSE showing distinctive staining (score 4) (×200). **e** Haematoxylin- and eosin-stained section showing vacuolation (score 2) in the parietal cortex of goat JO954 with terminal disease 359 days following inoculation with CH1641 (×200). **f** Nil immunohistochemical staining of PrP with BG4 in the parietal cortex of goat JO954 with terminal disease 359 days following inoculation with CH1641 (×200). **g** Haematoxylin- and eosin-stained section showing vacuolation (score 2) in thalamic nuclei from goat 45×45 with terminal scrapie 547 days following intracerebral inoculation with BSB (×200). **h** Immunohistochemical staining with BG4 for PrP in hypothalamic nuclei of goat 45×45 with terminal disease 547 days following inoculation with BSE showing staining (score 2) (×200) [Reproduced from Foster et al. (2001) with kind permission of the publisher of the Journal of Virology]

homeostasis; and (4) the immune system. The prion-induced modification in Ca^{2+} homeostasis is the result of: (1) prion interaction with intrinsic ion transport proteins, e.g. L-type Ca^{2+} channels in the surface membrane and IP_3-modulated Ca^{2+} channels in the internal membranes; and/or (2) formation of cation channels by PrP itself. These two mechanisms of action lead to changes in Ca^{2+} 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 Ca^{2+} homeostasis (which does not exclude changes in endogenous Ca^{2+} transport pathways and Cu^{2+} 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 PrP^{c} isoform to the predominately β-sheet PrP^{Sc} isoform. This conformational change suggests that the β-sheet region plays an important part in the PrP^{Sc} cytotoxicity. Also, most of the pathogenic characteristics of PrP^{Sc} 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 PrP^{Sc}, which explains the use of PrP[106–126] as a tool to characterize the pharmacological and biophysical properties of PrP^{Sc}. It is thought that PrP^{Sc}-induced cell death could be mediated via changes in Ca^{2+} 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 PrP^{Sc} 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 Ca^{2+} 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 Ca^{2+} 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_{Na}/P_{Cl}, of $\sim$2.5. However, the presented data suggest that the current flow is

through several heterogeneous ion channels. We have confirmed that PrP[106–126] forms single ion channels as shown in Fig. 2 (Kourie and Culverson 2000; Kourie et al. 2001). Based on the conductance and kinetic parameters of the single channel currents recorded in 250/50 mM KCl *cis*/*trans*, we have found that PrP[106–126] formed heterogeneous cation channels that differ in their conductance and kinetic properties. The most frequently observed PrP[106–126]-formed single cation channels were those of: (1) a GSSG- and TEA-sensitive channel with fast kinetics; (2) a DTT-sensitive channel with slow kinetics; and (3) a large channel. In addition, the differences in the response of these channel types to TEACl, GSSG, DTT, SO_4^{2-} and Zn^{2+} suggest that these channels differ in their pharmacological properties.

Age-related derivatives, PrP[106–126] (L-Asp108) and PrP[106–126] (L-iso-Asp108), of the prion protein fragment 106–126 (PrP[106–126] (Asn108)) (Sandmeier et al. 1999) also form heterogeneous ion channels. These deamidated isoforms showed no enhanced propensity to form heterogeneous channels compared with PrP[106–126] (Asn108), which is in agreement with the findings of Kagan and co-workers (Lin et al. 1997). One of the PrP[106–126] (L-Asp108)- and PrP[106–126] (L-iso-Asp108)-formed channels had three kinetic modes (Fig. 2). The biophysical properties of PrP[106–126] (L-Asp108)- and PrP[106–126] (L-iso-Asp108)-formed channels and their response to Cu^{2+} were similar to those of channels formed with PrP[106–126] (Asn108) (Kourie and Culverson 2000; Kourie et al. 2001).

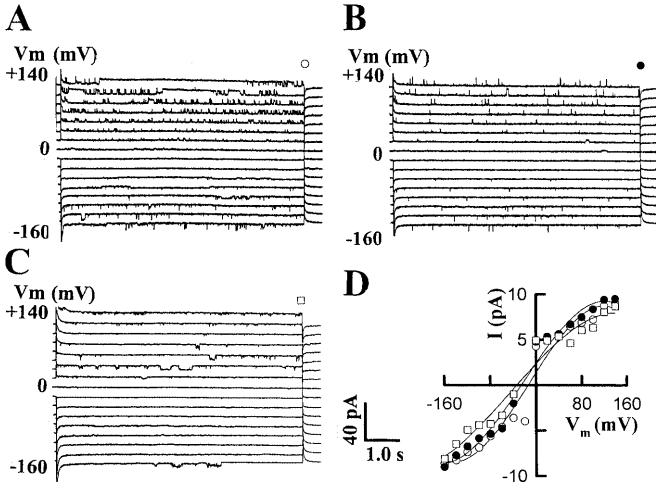

Fig. 2A–D. Representative families of current traces illustrating the kinetic modes of a deamidated isoform of prion PrP[106–126] (L-AsP108)-formed cation channel in an optimal bilayer clamped at different V_m values between –160 and +140 mV in KCl (250 mM/250 mM; *cis*/*trans*). **A** Mix of fully open and transiently open channel mode; **B** transiently open channel mode; and **C** fully open channel mode. Following convention, the upward deflections denote activation of outward ion current. For a better display, the data are filtered at 1 kHz, digitized at 2 kHz and reduced by a factor of five. The current traces are separated by a 10 pA offset. **D** Current-voltage relationships for the mixed fully open and transiently open channel mode (*open circles*), transiently open channel mode (*solid circles*) and fully open channel mode (*open squares*). The *solid lines* are drawn to a third-order polynomial fit

Molecular mechanism for the formation of heterogeneous channels

The cellular responses to this peptide PrP[106–126] (and PrP^{Sc}) are complex and depend on its physical state (Kunz et al. 1999; Sandmeier et al. 1999). Bilayer studies indicate that it is very likely that the peptide's conformation and propensity to aggregate into fibrils is enhanced in the lipid environment. The precise molecular mechanism for the formation of heterogeneous channels is not known. The formation of heterogeneous channels could be due to: (1) different isoforms, (2) different levels of peptide aggregation, (3) different degrees of conversion, or (4) a combination of these factors. The diversity of the PrP[106–126]-formed ion channels is in agreement with the concept of conformational diversity, resulting from the possibility of conversion to different tertiary structures of PrP^{Sc} (Prusiner et al. 1998). The concept of PrP[106–126] forming different aggregated pathogenic ion channels is also consistent with the hypothesis that, during internal shuttling, PrP may face different conditions which aggregate the PrP into β-sheets or fibrils. This PrP aggregation is characteristic of other intrinsically toxic amyloid-forming peptides (Manuelidis and Fritch 1996). Electrophysiological experiments support the role of amyloid peptide aggregation in the formation of a non-selective ion channel, which was indicated by the findings that disruption in the aggregation of amyloid AβP1–42 channels with Congo Red treatment prevented the formation of this channel (Hirakura et al. 1999). Acidic solutions also enhance peptide aggregation and β-sheet formation (De Gioia et al. 1994) and ion channel formation (Lin et al. 1997). The formation of functionally different pathologic ion channels is also in agreement with the possibility that apoptosis and induction of hypertrophy and proliferation might also be regulated by the alternative structures of proteins.

There is no reason to suspect that prion isoforms cannot form ion channels in vivo. On the contrary, there is evidence for the prion-induced membrane damage and for prion-formed channels. Kawahara et al. (2000) found that PrP[106–126], like AβP and human islet amylin, induced an increase in the intracellular free Ca^{2+}, through Ca^{2+} permeable channels, of the immortalized hypothalamic GnRH neurons (GT1–7 cells), as measured with the fura-2 technique. They proposed that the unregulated Ca^{2+} entry via peptide-formed channels could be a common mechanism causing cell death. It is hypothesized that changes in the structure and composition of the prion peptide leads to changes in the β-sheet-based prion, enhancing its vacuolation of the endoplasmic reticulum, and this is very likely to be followed by further interactions with the mitochondrial

and nuclear membranes. Such a mechanism plays a major role in prion's ability to destabilize and vacuolize cell membranes. The change in the intracellular pH is one of the conditions that appears to play a role in protein misfolding and channel formation.

It has recently been proposed that the transmembrane protein ^{Ct}mPrP represents a common step in the pathogenesis of genetic and infectious diseases (Hegde et al. 1999). However, it remains to be seen how ^{Ct}mPrP affects the intercellular pathways leading subsequently to neurodegenerative diseases. It will be very interesting to examine the properties of the ion channels that could be formed with ^{Ct}mPrP. This may help to differentiate the type of ion channels that cause pathology from those that are benign or less effective pathological channels. The type of channel and its potency to cause pathology may explain why the accumulation of PrP^{Sc} is not the sole cause of pathology (Tateishi et al. 1990; Hsiao et al. 1994) and the time course of its accumulation does not match the course of neurodegeneration (Manson 1999). What appears to be an essential conversion of PrP^{Sc} to ^{Ct}mPrP may, in fact, represent the transformation of the PrP^{Sc} conformation to a pathogenic channel configuration. It may be that ^{Ct}mPrP has that pathogenic ion channel configuration and/or it is more fusogenic than other PrP configurations. The concept of ''breech-birth properties'' (Hope 1999) is in agreement with the concept of PrP interaction with membranes and the formation of channels as a mechanism of membrane damage underlying prion pathologies. The findings that PrP[106–126] forms different ion channels is consistent with the findings that PrP synthesized in cell-free translation systems can be found in more than one topologic form: ^{Ct}mPrP, ^{Nt}mPrP and ^{sec}PrP (Hegde et al. 1998). These topologic PrP forms have different fusogenic properties. The ^{Ct}mPrP, which has been proposed as necessary and sufficient for the development of disease (Hegde et al. 1999), is thus a transmembrane topologic form that is essential for the formation of the pathogenic ion channel configuration and/or more fusogenic than other PrP configurations. The hypothesis that a specific transmembrane protein (C but not N) is consistent with a specific pathogenic ion channel, i.e. not every peptide that interacts with the membrane is harmful and, furthermore, not every transmembrane-forming ion channel can cause membrane damage. Furthermore, recent findings (Gu et al. 2002) show that PrP[106–126] induces accumulation of PrP^{c}, which is a requirement for PrP[106–126]-induced toxicity (see Singh et al. 2002 and references therein). The findings of Gu et al. (2002), which suggest that PrP[106–126] acts by inducing the accumulation of membrane-associated PrP^{c}/^{ctm}PrP, are in agreement with prion channel model. According to the prion channel model, accumulation of the transmembrane prion protein ^{ctm}PrP is part of a mechanism that leads to the formation of a neurotoxic ion channel.

Although the precise molecular mechanism for PrP[106–126] channel formation is not known, it is very likely that the interaction between the negatively charged membranes and positively charged lysine residues plays a role in vivo. Such interactions may enable the membranes to be involved in PrP^{Sc} aggregation, orientation and configuration to form ion channels. It is known that conversion of normal cellular prion protein PrP^{C} to the pathologic prion protein PrP^{Sc} involves conversion of α-helical and coil structures to a β-sheet structure. It is also known that β-sheet formation on membranes has been implicated in the initiation of β-amyloid fibril formation and neurotoxicity. This may suggest that only β-sheet-based channel types could be significant in prion pathology. In accordance with the pore-forming toxins, e.g. anthrax toxin, that have β-sheet-based structures, Hirakura et al. (1999) proposed that PrP[106–126] forms β-sheets, which aggregate prior to the formation of a channel whose structure is thought to be that of a β-barrel. A model for such β-barrel pathogenic prion channels has been proposed recently (Chapron et al. 2000). Also recently, Wille et al. (2002) used electron crystallography to study the structures of two infectious variants of the prion protein, the N-terminally truncated PrP^{Sc} and a miniprion (PrP^{Sc}106). Two-dimensional crystal structure models featuring parallel β-helices were constructed for PrP^{Sc}. The models show trimeric dimers and trimeric symmetry. Interestingly, these findings are in close agreement with the structural computations proposed for model PrP^{Sc} channels (Chapron et al. 2000) and thus provide further support for the prion channel model.

Pathophysiological significance of PrP[106–126]-formed channels

The diversity of the prion-formed channels suggests that multi-molecular species could be involved in cytotoxicity, although some results indicate that some of the commonly observed aggregates, e.g. fibrils, may not be among them. A model of cell damage by a single channel type would not encompass all possible aggregates; however, it must also be emphasized that not every PrP-formed channel observed in vitro will necessarily have the same pathological significance. The characterization of PrP[106–126] aggregates and their corresponding formed channels may also shed light on the contradictory findings concerning the lack of toxicity of PrP[106–126] (Kunz et al. 1999).

Loss of cellular membrane compartmentalization

The cytotoxic activity of PrP[106–126] is believed to result from an interaction of this peptide with the components of cell membranes (lipids and proteins), leading to changes in membrane permeability and

disruption of the ionic gradients across the membrane. PrP[106–126] does this via the formation of ion channels in the membrane and/or by an increase in membrane permeability by acting on ion channels already found in the membrane. PrP[106–126] may exert its effects, by mutually non-exclusive mechanisms, on (1) the lipid environment and/or (2) signaling and ion transport proteins. Additionally, PrP[106–126] may form different aggregates, each of which per se exerts its action on the membrane by mutually non-exclusive mechanisms. The formation of different aggregates is very likely the result of different degrees of β-sheet formation. It is suggested that in vivo aggregation and ion channel formation underlies vacuolation of intracellular organelles.

Membrane compartmentalization is an early evolutionary step and the regulation of ion transport across these cellular compartments is fundamental for cell survival. The PrP[106–126]-induced changes in the V_m are important to cell function: these ionic currents are expected to affect voltage-dependent mechanisms of neurons. The low selectivity of the large conductance cation of the PrP[106–126]-formed channels suggests that these channels may modify V_m and the electrolyte homeostasis by dissipating K^+ and Cl^- gradients across the cellular membranes. These changes could mediate the pathophysiological function(s) of the PrP[106–126]-formed heterogeneous cation channels. In agreement with previous findings regarding the role of Cu^{2+} and Zn^{2+} in the aggregation and neurotoxic properties of PrP[106–126] (Jobling et al. 2001), PrP[106–126]-formed channels are sensitive to Cu^{2+} and Zn^{2+} and reducing and oxidizing agents (Kourie and Culverson 2000; Kourie et al. 2001), suggesting that they could be modulated by changes in the homeostasis of the brain's transitional metals and the redox state of the cell. The action of these metals, which have been linked to neurodegenerative diseases, might be due to their ability to modify the secondary structure and enhance aggregation of the prion proteins and their action on membranes.

Vacuolation mechanism

In addition to the ability of prion-formed ion channels to induce loss of signaling and electrolyte homeostasis, ion channel-forming proteins could affect cell function by inducing vacuolation, which is a non-specific response to pathologies associated with the malfunction of epithelial, muscular and neuronal cells. The loss of cellular membrane compartmentalization and the associated changes in second messenger and electrolyte homeostasis precede vacuolation. Ultrastructurally, neurodegenerative-associated vacuoles could be bound by a single membrane and may contain granular and membranous materials (Kortz et al. 1997). Such vacuolation is thought to be one of the histological symptoms, together with astrocytosis, protein aggregation, fibrilization and amyloid deposition, that are the hallmarks of the loss of neurons in prion diseases. The characteristics of these vacuoles, that are often located within dendrites and axons, have been used for classification of strains of scrapie and prion agents in bovine spongiform encephalopathy and variant Creutzfeldt-Jakob disease (Betmouni et al. 1999). The physiopathological significance of these prion-induced structures is not well established. However, PrP^{Sc} accumulation has been linked to neuronal membrane dysfunction and clinical features of prion diseases that have been explained by altered ion channel function, secondary to decreased plasma membrane fluidity (DeArmond et al. 1996; Wong et al. 1996). According to this argument, such a mechanism has the potential to functionally disconnect neuronal networks and cause neuronal vacuolation. It has also been noted that membranous whorls and multicompartment vacuoles are often pathological hallmarks of protein-induced cell death across species (Garcia-Anoveros et al. 1998). The alternative that we propose to PrP^{Sc}-induced changes in intrinsic channels and membrane fluidity is the PrP^{Sc} formation of channels as a pathogenic mechanism, linked with PrP^{Sc} accumulation and to plasma membrane abnormalities in prion diseases, as has also been considered by several laboratories (Lin et al. 1997; Kawahara et al. 2000; Kourie and Culverson 2000). We have advanced the hypothesis that prion isoforms can, in addition to their ability to modify intrinsic ion transport pathways and membrane properties, potentially form diverse ion channels affecting fluidity of the plasma membrane and its functions (Tagliavini et al. 2001; Kourie 2001). Interlinked changes in membrane fluidity and endogenous ion channels induced by prion isoforms can occur independently and concurrently with ion channel formation, i.e. they are not mutually exclusive. PrP^{Sc} accumulation alters the ability of chaperones to correctly fold plasma-membrane proteins during their synthesis, which directly affects the properties of nascent proteins and, secondarily, affects membrane fluidity. Several ion channel-forming toxins and misfolded cytotoxic proteins induce vacuolation. Prion, which has a channel-forming hydrophobic region (Lin et al. 1997; Kawahara et al. 2000; Kourie and Culverson 2000; Kourie et al. 2001) induces vacuolation. Other ion channel-forming proteins which induce vacuolation include the well-established VacA of *Helicobacter pylori* (Czajkowsky et al. 1999; Iwamoto et al. 1999; Szabo et al. 1999; Tombola et al. 1999a, 1999b, 2000), Vero cells (Figueroa-Arredondo et al. 2001) and aerolysin (Abrami et al. 1998). Similarly, there are other examples of vacuolation, such as melittin-induced micellization and membrane fusion as an antibacterial mechanism. These observations point to the similarities and significance of prion-induced vacuolation and AβP-induced endocytosis in neurodegenerative disease. We propose that ion channel formation is a part of the molecular mechanisms responsible for

vacuolation, associated with prion disease. We further propose that initial vacuolation could be an adaptive cellular response to compartmentalize the pathogenic prion. However, as the concentration of pathogenic prion increases, the ability of the cell to compartmentalize these prions in vacuoles declines. These suggestions are in agreement with the proposals that: (1) vacuolation is a secondary non-specific spongiform-induced change, following a disruption of axonal transport (Jeffrey et al. 1992); (2) vacuolation is triggered by the accumulation of PrP^{Sc} or other pathogenic conformations within the lysosomal compartment; and (3) at later stages the degree of vacuolation may not be proportional to the accumulation of lipid, e.g. in mouse, scrapie does not correspond to the distribution of vacuoles. The mechanism of the formation of these vesicles is not known. The deposit of lipids or carbohydrate and extracellular matrix materials have been invoked as a source for the formation of these vacuoles (Betmouni et al. 1999).

There is also pharmacological evidence linking vacuolation to ion transport pathways. The vacuolation that is induced by *V. cholerae* is exacerbated by agents that block vacuolar proton pumping and it is suggested that vacuolation contributes to the virulence of *V. cholerae* strains by damaging intracellular membrane trafficking or ion exchange in target cells (Figueroa-Arredondo et al. 2001). It will be interesting to examine whether prion-associated vacuolation can be inhibited by ion channel blockers, which have been reported to reverse some of the effects of prion diseases, as well as antimalarial and antipsychotic drugs. There is further evidence showing VacA-induced vacuolation and that VacA channels can be inhibited by known chloride channel blockers, e.g. 5-nitro-2-(3-phenylpropylamino)benzoic acid (NPPB), *N*-phenylanthranilic acid, diethyl pyrocarbonate, and 4,4′-diisothiocyanatostilbene-2,2′-disulfonic acid (DIDS), which can also inhibit and partially reverse vacuolation of HeLa cells (Tombola et al. 2000). It is thought that channel activity may contribute to vacuolation through the increase in anion permeability, causing a rise in osmotic pressure in acidic compartments within the cell into which the toxin is internalized. This causes osmotic swelling, leading to the formation of large vacuoles. Channel formation may allow further fusion into membranes. For example, VacA synthesized in the cytoplasm may enter the endosomal membrane directly from the cytoplasm in the form of vacuoles. The role of cytosolic factors, e.g. redox state, second messengers and copper, which have been shown to modulate prion-formed channels in vacuolation, also needs to be investigated. There is some evidence linking such cytosolic factors to vacuolation (Yoshikawa et al. 1996; Kim et al. 2000; Wilt et al. 2000). It has also been suggested that iron metabolism is changed and that iron-induced oxidative stress partly contributes to neurodegeneration in scrapie infection (Kim et al. 2000). In addition, enzootic ataxia-induced pathological changes in Sika deer, such as spongy vacuolation, were linked to low copper concentrations in the serum and liver, suggesting that decreased activity of copper-containing enzyme induced vacuolation (Yoshikawa et al. 1996). It is very likely that this would be mediated by structural changes that affect prion interaction with membranes.

Vacuolation in cellular organelles within the central nervous system is a common cellular response associated with neurodegeneration. The dysfunction of the membranes of internal organelles is a consequence of channel formation by an excess of misfolded proteins, incorporated into the membranes of these organelles. In the case of prion disease the spongiform change is caused by an excess of unchaperoned, stray PrP^{Sc} that needs to be compartmentalized. However, the hydrophobic regions of the misfolded, abnormal protein lead to the formation of channels, causing membrane dysfunction. This is followed by the leakage of the compartmentalized PrP^{Sc}, which induces further membrane damage.

Pharmacological agents which modulate prion-formed channels have the potential to be used to rectify the cytotoxic effects of these peptides in vivo. More importantly, before ion channel formation with prion occurs, specific pharmacological agents, e.g. β-sheet breakers, could be exploited for prevention of both pathological prion conformations and of prion aggregates that underlie channel formation and amyloidogenesis. If PrP^{Sc} or any other isoforms and fragments form heterogeneous ion channels in a manner similar to that of PrP[106–126], then the diverse kinetics of such channels, in addition to their heterogeneity as a result of self-propagating, conformational differences, suggest different mechanisms of cytotoxicity and, hence, multiple therapeutic approaches.

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