Channels formed with a mutant prion protein PrP(82–146) homologous to a 7-kDa fragment in diseased brain of GSS patients

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Submitted 26 February 2003; accepted in final form 23 May 2003.

Summary

PRION PROTEIN (PrP)-related encephalopathies are characterized by the accumulation of pathogenic forms of the PrP, termed PrP scrapie (PrPSc), in the central nervous system. Unlike the normal cellular isoform (PrPc), PrPSc has a high content of β-sheet secondary structure, is insoluble in non-denaturing detergents and partially resistant to protease digestion, and has the propensity to form amyloid fibrils. Synthetic peptides of different PrP regions have been useful in structure-function relationship studies, e.g., identifying the function of different PrP domains in the conformational transition of PrPc to PrPsc and in prion pathogenesis (51, 52). It has been shown that the synthetic PrP(106–126) possesses many of the PrPSc properties, e.g., propensity to adopt a β-sheet conformation, formation of amyloid fibrils, partial resistance to proteinase K (PK) digestion, and the ability to induce nerve cell degeneration and glial cell activation in vitro (13, 51). In addition to its high fibrillogenic properties and toxicity to neurons in vitro (8, 13, 48, 51), it has the ability to form ion channels (21, 25, 27, 34). The highly hydrophobic segment AAAAAAGA from positions 113 to 120 (51) confers on the fragment the ability to interact with lipid membranes and form ion channels (29). The toxic effects of PrP(106–126) on neuronal cells and its ability to induce apoptosis (8, 13, 45, 61), as well as its ability to mimic the toxicity of PrPTESE (transmissible spongiform encephalopathic PrP) to cause cell death (8, 14, 20, 47), have been the given justifications for its use as an investigative tool in PrP research. However, an important argument against the significance of data obtained using synthetic PrP(106–126) fragment are the facts that it is not occurring naturally and it is not from a pathogenic mutant PrP. Thus it has been suggested that it is important to use naturally occurring longer fragments or full-length PrP mutant to validate the findings obtained using PrP fragments such as PrP(106–126). A major PrP mutant fragment that forms amyloid fibrils in the diseased brain of Gerstmann-Sträussler-Scheinker syndrome (GSS) patients is a PrP fragment of 7 kDa that spans from residues 81–82 to 144–153 of PrP (see Refs 52 and 53). This fragment is very similar in

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patients with different mutations (e.g., A117V, P108S, Q217R) and is derived from mutant PrP. PrP(82–146) kills cells but does not replicate in animals and is not infectious or transmissible (Salmona M, Morbin M, Massignan T, Colombo L, Mazzoleni G, Capobianco R, Diomede L, Thaler F, Mollica L, Musco G, Kourie JI, Bugiani O, Sharma D, Inouye H, Kirschner DA, Forloni G, and Tagliavini F, unpublished data).

In this study we have examined the ability of synthesized PrP(82–146) (Fig. 1) to form ion channels that may explain the neurotoxicity of this fragment. We report that PrP(82–146)-formed channels possess conductance and kinetics properties and Cu2+ sensitivity similar to those of PrP(106–126)-formed channels, indicating that the longer PrP fragment maintains the configuration(s) for channel formation and that the region 106–126 is essential in the formation of these channels. We propose that the PrPSc may form such ion channels, which could be part of its neurotoxic mechanism.

MATERIALS AND METHODS

Peptide synthesis. The following four peptides were used for the study: 1) PrP(82–146) WT (wild type), GzzQPHGGGWGQGGGTHSQWNKPSKPKTNMKHMAGAAAAGAVGGGLGGYMLGSAMSRPIIHFGSDYE; 2) PrP(82–146) (106–126) SC (with a scrambled sequence in the region spanning residues 106–126), GzzSPHGGGWGQGGGTHSQWNKPSKPKNGAKALMGHGATKVGMGAAYLMGLSAMSRPIIHFGSDYE; 3) PrP(82–146) (127–146) SC (with a scrambled sequence in the region spanning residues 127–146), GzzQPHGGGWGQGGGTHSQWNKPSKPKTNMKHMAGAAAAGAVGGGLGGYMLGSAMSRPIIHFGSDYE; and 4) PrP(82–146) SC (with a totally scrambled sequence), EzzSADQFALGGSKHNGMQVQVAGHGGSMSGMAGAKAWNGHPSGTG1PTAKVMVPKYGGGWAGMGRPSS.

Peptides were synthetized in laboratories by M. Salmona using solid-phase synthesis on a model 433 synthetizer (Applied Biosystems, Foster City, CA) with the use of N-(5-fluorenyle) methoxycarbonyl as the protective group for the synthesis of residues and of the solvent drain every amino acid. Peptides were cleaved from the resin with a mixture of phenol-thioanisole-ethandiol-trifluoroacetic acid (TFA), precipitated with cold diethyl ether, and washed several times with the same solvent.

Peptide purification was carried out using a semipreparative column (Delta Pack C18 column; 190 × 300 mm, 300-Å pore size, 15-μm particle size; Nihon Waters, Tokyo, Japan) with an HPLC apparatus (model 243; Beckman Instruments, Palo Alto, CA) equipped with a detector set at 214 nm (model 160; Beckman Instruments). A linear gradient of 100% water containing 0.1% TFA to 80% acetonitrile containing 0.08% TFA over 60 min was used with a flow rate of 3 ml/min. The fractions containing PrP fragments were separately collected, lyophilized, and kept at −80°C.

The purity and the composition of peptides were determined by amino acid sequencing using a 46600 Prosequencer (Milligen, Bedford, MA) and electrospray mass spectrometry (model 5989A; Hewlett-Packard). The instrument was calibrated with either horse skeletal muscle myoglobin or a synthetic peptide with a known molecular weight. The electrospray potential was −9 kV, and the quadrupole mass analyzer was set to scan over a range from m/z = 750 to m/z = 1350 Da; the molecular weights were calculated from several multiple-charged ions within a coherent series. The samples used for calibration were dissolved in a solution of water-methyl alcohol (1:1) containing 1% acetic acid (vol/vol) to obtain a final concentration of ~2 pmol/μl. The samples were injected directly into the ionizing chamber at a flow rate of 2 μl/min.

Lipid bilayer technique. Bilayers were formed across a 150-μm hole in the wall of a 1-ml Delrin cup using a mixture of palmityl-oleoyl-phosphatidylethanolamine (PE), palmitoyl-oleoyl-phosphatidyserine (PS), and palmitoyl-oleoyl-phosphatidylcholine (PC) (30, 36), obtained in chloroform from Avanti Polar Lipids (Alabaster, AL). It has been suggested that the membranes can play a role in PrPSc dimerization through biasing the orientation and configuration of the PrPSc, which is enhanced by the lysine residues 101, 104, and 106 (60). To exclude the role of the variations arising from different membrane compositions in the formation of distinct PrP(82–146) channels, we recorded ionic currents from bilayers made of a single lipid mixture, i.e., PE:PS:PC (3:3:2 by volume). The lipid mixture was dried under a stream of N2 gas and redissolved in n-decane at a final concentration of 50 mg/ml. Synthetic PrP(82–146), which has three amino acids with a net charge of −2 at pH > 7 (obtained from Bachem, Bubendorf, Switzerland), was dissolved in 250 mM KCl, divided into aliquots, and kept at −84°C until use. The side of the bilayer to which the peptide or liposomes were added was defined as cis and the other side as trans. The peptide was incorporated into the negatively charged lipid bilayer by addition of microliter aliquots of the dissolved peptide to the cis chamber at a final peptide concentration of 0.1–1 μg/ml. Ion channels were also recorded from a peptide-lipid mixture of 1:50. Unless stated otherwise, the initial experimental solution for incorporating synthetic PrP(82–146) into the bilayers contained KCl (250 mM cis/50 mM trans), 1 mM CaCl2, and 10 mM HEPES and was adjusted to pH 7.4 with KOH. The experiments were conducted at 20–25°C.

Preparation of liposomes. In some experiments, liposomes of PrP(82–146) and its mutants were used. The method for the preparation of these liposomes was described by Arispe et
RESULTS

Synthesis of PrP(82–146) peptides. To determine the ability of the infectious PrPSc to form ion channels, we used a synthesized PrP mutant fragment and isofoms (53). This approach was used for 1) ascertaining the significance of the PrP(106–126) and its hydrophobic segment in channel formation and 2) determining the contribution of other domains of PrP(82–146) to the types and regulation of the formed channels. The four PrP(82–146) mutants that contain the membrane-spanning domain (residues 113–135) of PrP (see Refs. 16 and 57) were synthesized and incorporated into lipid bilayers as detailed in MATERIALS AND METHODS.

Ion channel recording. The pCLAMP6 program (Axon Instruments) was used for voltage command and acquisition of ionic current families with an Axopatch 200 amplifier (Axon Instruments). The current was monitored with an oscilloscope, and the data were stored on a computer. The cis and trans chambers were connected to the amplifier head stage by Ag-AgCl electrodes in agar salt bridges containing the solutions present in each chamber. Membrane voltages (Vm) and ionic currents were expressed relative to the trans chamber.

Data analysis. Modifications in the bilayer thickness, mediated via lipid solvents, e.g., n-decane, contribute to changes in channel gating kinetics of ion channel (22), particularly those formed with short peptides, e.g., the 15-amino acid gramicidin-formed channel (38). Therefore, standardizing the specific membrane capacitance (Cm) and its time independence is important in comparative and detailed investigations of ion channel characteristics. Kinetic analysis for the PrP(82–146)-formed channels was conducted only for optimal bilayers having a Cm of >0.42 pF/cm2 and containing a single active channel. The criteria for defining ionic currents as belonging to a "single channel" have been described elsewhere (11). Single-channel activity was analyzed for overall characteristics using the program CHANNEL 2 (developed by Gage PW and Smith M, see Ref. 30). The following kinetic parameters of single-channel activity (32- to 128-s-long records) were determined: mean open time, T0 (i.e., the average of the open times of all intervals where the current exceeded the baseline noise for 0.5 ms); frequency of opening to all conductance levels, F0; and open probability, P0 (i.e., the sum of all open times as a fraction of the total time). CHANNEL 2 also allows online analysis of the entire current record for computation of the maximal current (I) and mean current (I0). I is defined as the integral of the current passing through the channel divided by the total time. The integral current is determined by computation of the area between a line set on the noise of the closed state and channel opening to various levels. The threshold level for the detection of single-channel events was set at 50% of the maximum current (42). The maximal current (I) is the current amplitude of a fully open channel. The maximal current was obtained by measuring the distance (in pA) between two lines, one set on the noise of the closed level where the current amplitude is 0 pA and the other set on the noise of the majority of distinct events that were in the open state. The maximal current was also obtained by measuring the distance (in pA) between the peak at 0 pA (representing the closed state) and the extreme peak on the right (representing the open state) in the all-point histogram generated using CHANNEL 2 (see Ref. 30). Both methods were used, and the results were generally in agreement. The current reversal potential (Em) was corrected for ionic mobility and liquid junction potential (2). Each channel was used as its own control, and the comparison was between conductance and kinetic parameters of the same channel recorded at different Vm before and after the channel was subjected to any treatment. Data are reported as means ± SE, and the difference in means was analyzed by using Student’s t-test. Data were considered statistically significant when P values were ≤0.05.
nels, were classified to 1) outward current with fast kinetics, 2) inwardly directed current with fast kinetics, 3) outwardly directed irregular “spiky” current, and 4) time-dependent inactivating current (Fig. 2; see also Ref. 29).

Current-voltage relationships were constructed to examine the voltage dependence of the conductance of the PrP(82–146) WT channel types (Fig. 3A). The current-voltage relationship of the time-dependent inactivating current was fitted with a third-order polynomial. The $E_{rev}$ of this channel type was between −6 and −11 mV, indicating that under these experimental conditions, the time-dependent inactivating current was due to the movement of $K^+$ and some $Cl^-$. The values of the $E_{rev}$ for the other channel types were between −24 and −35 mV, indicating that these currents were primarily due to the movement of $K^+$ (see also Fig. 9A). To confirm the time-dependent channel inactivation, we constructed the ensemble average of the currents of seven episodes at −140 and +140 mV (Fig. 3B). The data show that the channel at −140 and +140 mV tended to inactivate, and the inactivation of the currents could be described by a third-order polynomial fit. The voltage dependence of the inactivation for the time-dependent inactivating channel was examined by plotting the ratio $I_m/I_i$, where $I_m$ is the steady-state current at the end of the voltage step and $I_i$ is the initial current activated immediately after the voltage step, for different voltages (Fig. 3C). The solid line is a third-order polynomial fit of the data. The inactivation ratio decreases when the membrane is made either more positive than +80 mV or more negative than −80 mV.

Although PrP(82–146) WT (Fig. 2) and PrP(82–146) (127–146) SC (Fig. 4) elicit all four ion channel types, it appears that the outwardly directed current with fast kinetics is associated with PrP(82–146) WT, whereas the time-dependent inactivating current is associated with PrP(82–146) (127–146) SC. PrP(82–146) peptides in which the region from residue 106 to 126 had been scrambled, e.g., PrP(82–146) SC and PrP(82–146) (106–126) SC, showed a reduction in interaction with lipid membranes and did not form ion channels. The distribution of PrP(82–146) WT-formed channels included 41.2% fast kinetic and 11.5% time-dependent inactivating channel types, whereas the PrP(82–146) (127–146) SC-formed channels included only 18.6% fast kinetic and 68.6% time-dependent inactivating channel types, respectively. These findings suggest that an increase in PrP length does not prevent the 106–126 region from interacting with membranes and forming ion channels.

To ascertain the Cu$^{2+}$ sensitivity of PrP(82–146) WT- and PrP(82–146) (127–146) SC-formed ion channels in a way that is similar to those reported for the PrP(106–126)-formed channels (27, 29), we examined the effects of Cu$^{2+}$ on the formed channels. We found that the PrP(82–146) WT fast cation channel is Cu$^{2+}$ sensitive (Fig. 5, A and B). The kinetic parameters describing the channel activity were also obtained at voltages between −160 and +140 mV and in the absence and presence of 50–300 μM [Cu$^{2+}$] (data not shown). Cu$^{2+}$ modified the kinetic parameters of the fast cation channel in the burst mode. At positive voltages between 0 and +140 mV, Cu$^{2+}$ decreased the values of $P_o$ and $T_o$ and increased the values of $P_c$ and mean closed time ($T_c$). For example, at $V_m$ of +140 mV, 200 μM [Cu$^{2+}$]$_{cis}$ decreased the mean values of $P_o$ and $T_o$ from 0.63 and 7.8 ms to 0.17 and 1.8 ms, respectively, and increased the values of $P_c$ and $T_c$ from 67 events per second and 3.2 ms to 134 events per second and 9.4 ms, respectively. The Cu$^{2+}$-induced voltage-dependent changes in $P_o$ were primarily due to decreases in the values of $T_o$ and increases in $T_c$. The findings are in agreement with the effects of Cu$^{2+}$ on

Fig. 2. Ion channel types indicated from membrane current activity elicited with wild-type PrP(82–146) WT peptide. Outwardly directed current with fast kinetics (B), outwardly directed irregular current (C), and time-dependent inactivating current (D) were recorded from voltage-clamped bilayers at membrane voltages ($V_m$) between −160 and +140 mV in KCl (250 mM cis/50 mM trans). Following convention, the upward deflections denote activation of outward K$^+$ current, i.e., K$^+$ moving from the cis chamber to the trans chamber. For a better display, the data have been filtered at 1 kHz, digitized at 2 kHz, and reduced by a factor of 5. Current traces are offset by 10 pA in A–C and by 120 pA in D.
the kinetics of PrP(106–126)-formed fast cation channels that have been fully detailed by Kourie et al. (29).

To deduce the role of the \( \beta \)-sheet in the formation of this channel type, we examined the effects on the PrP(82–146) WT-formed channels of rifampicin (RIF), an antibiotic against leprosy, which has been reported to inhibit the anti-parallel \( \beta \)-sheet-based aggregation of amyloid \( \beta \) peptide (A\( \beta \)) and to reduce neurotoxicity (54, 56). The \( \beta \)-sheet-based aggregation of PrP, like that of A\( \beta \), is a channel-forming configuration (23). The action of RIF is by an unknown mechanism, other than reactive oxygen species production, which has been proposed to underlie human amyloidosis (see Refs. 5 and 18). We found that the PrP(82–146) WT-formed cation channel is RIF insensitive (Fig. 5, C and D).

In agreement with previous findings (27), we found that unlike the PrP(82–146) WT and PrP(82–146) (127–146) SC fast cation channel, the time-dependent inactivating channel is neither Cu\(^{2+}\) (Fig. 6, A and B) nor RIF sensitive (Fig. 6, C and D). The time-dependent inactivating PrP(82–146) WT and PrP(82–146) (127–146) SC channels could not be blocked with Cu\(^{2+}\), even with concentrations as high as 750 \( \mu \)M in both cis and trans solutions (Fig. 6B). In addition, the current activity of the time-dependent inactivating channel could not be regulated with the reducing agent DTT (data not shown).

In contrast to the lack of 132 \( \mu \)M [RIF]\(_{\text{cis}}\) effects on the properties of fast kinetic cation channels already formed with either PrP(82–146) (127–146) SC or PrP(82–146) WT, in separate experiments PrP(82–146) WT channels formed in the presence of 100 \( \mu \)M RIF appeared electrically silent with the exception of a few muted transient opening and closing events (Fig. 7B), although channel opening to the different levels has been identified in long segments of recordings. Comparison between the current levels of two channels formed in the absence and presence of RIF indicates that the conductance levels C, O1, O2, O3, and O4, at 0, 2.2, 4.4, 6.6, and 8.8 pA, respectively, of the channel were not affected significantly (Fig. 7, A and B). Data analysis with CHANNEL 2 (30) revealed that RIF did not affect the single-channel conductance significantly. For example, at \( V_m \) of +140 mV, the amplitude of the maximal current \( I \) was 9.1 \pm 0.9 and 8.6 \pm 0.6 pA in the presence and absence of 100 \( \mu \)M RIF, respectively (\( n = 16 \) traces). On the other hand, the average mean current \( I' \) was reduced significantly, being 4.3 \pm 0.5 and 0.7 \pm 0.2 pA in the absence and presence of 100 \( \mu \)M [RIF]\(_{\text{cis}}\) (\( n = 16 \) traces).

To shed light on the role of another transitional metal linked to PrP diseases in protein channel aggregation, we probed the modulation by Cd\(^{2+}\) of the PrP(82–146) channels formed in the presence of RIF. The effects of [Cd\(^{2+}\)]\(_{\text{cis}}\) on the activity of an PrP(82–146) WT channel, formed in the presence of [RIF]\(_{\text{cis}}\), activated at \( V_m \) between –160 and +140 mV in KCl (250 mM cis/50 mM trans) was examined. Figure 8 shows the increase in the current transitions at positive voltages of an electrically semisilent channel after the cis addition of Cd\(^{2+}\) between 0.6 and 2.4 mM. Close inspection of the current amplitude and analysis with CHANNEL 2 (30) of current transitions to different current levels indicated that these are not the result of Cd\(^{2+}\)-enhanced channel activity of a single channel but, rather, the activity of several PrP(82–146) WT fast cation channels and that the incorporation of their subunits into the bilayer is enhanced with Cd\(^{2+}\).
Current-voltage relationships of the fast cation channel type at different [Cd\textsuperscript{2+}]\textsubscript{cis}. The current-voltage relationships constructed from ion current families, obtained at different voltages and concentrations of Cd\textsuperscript{2+}, show weak outward rectification of $I$ and $I'$ and confirm the steadiness of the current amplitude at depolarizing voltages (Fig. 9A). The amplitude $I$ and the weak current rectification were not affected by 0–2.4 mM [Cd\textsuperscript{2+}]\textsubscript{cis} (Fig. 9A). On the other hand, the physiologically important $I'$ was increased at both positive and negative voltages. The $E_{\text{rev}}$ for $I$ of the PrP(82–146) WT-formed fast cation channel in the presence of different [Cd\textsuperscript{2+}]\textsubscript{cis} were between −24 and −35 mV, depending on the quality of the polynomial fit, which are close to the $E_K$ at a value of −35.6 mV.

[Cd\textsuperscript{2+}] dependency of the fast cation channel current. The dose-response of the fast outward current transitions on [Cd\textsuperscript{2+}]\textsubscript{cis} was examined. $I$ and $I'$ were measured at several concentrations between 0 and 2.4 mM on PrP(82–146) WT. The [Cd\textsuperscript{2+}]\textsubscript{cis} was successively increased in 0.6 mM increments, and families of current traces were obtained at voltages between −160 and +140 mV. Figure 10 shows the Cd\textsuperscript{2+} dependence of $I'$ at voltages between +20 and +140 mV. The estimated concentration of Cd\textsuperscript{2+} that induces a 50% increase in $I'$ was between 1.45 and 1.86 mM for the voltages between +20 and +140 mV. The lack of a Cd\textsuperscript{2+} effect on the maximal current $I$, taken together with the Cd\textsuperscript{2+}-induced increase in $I'$, indicates that the

![Fig. 4. Ion channel types indicated from membrane current activity elicited with PrP(82–146) (127–146) SC peptide. Outwardly directed current with fast kinetics (A), inwardly directed current with fast kinetics (B), outwardly directed irregular current (C), and time-dependent inactivating current (D) were recorded from voltage-clamped bilayers at $V_m$ between −160 and +140 mV in KCl (250 mM cis/50 mM trans). Following convention, the upward deflections denote activation of outward K\textsuperscript{+} current, i.e., K\textsuperscript{+} ions moving from the cis chamber to the trans chamber. For a better display, the data have been filtered at 1 kHz, digitized at 2 kHz, and reduced by a factor of 5. Current traces are offset by 10 pA in A–C and by 120 pA in D.](http://ajpcell.physiology.org/)

![Fig. 5. PrP(82–146) WT-formed fast cation channel. A: control [Cu\textsuperscript{2+}]\textsubscript{cis} (0 Cu\textsuperscript{2+} in cis solution). B: 200 μM [Cu\textsuperscript{2+}]\textsubscript{cis}. C: control [RIF]\textsubscript{cis}. D: 132 μM [RIF]\textsubscript{cis}. Current traces are offset by 10 pA.](http://ajpcell.physiology.org/)
kinetic properties of this channel type are more sensitive than its conductance properties to increases in \([\text{Cd}^{2+}]_{\text{cis}}\), confirming the sensitivity of the channel to transitional metals as has been reported previously (27, 29).

**DISCUSSION**

**PrP(82–146) heterogeneous channels.** The main finding of this study is that synthesized, naturally occurring, long PrP(82–146) mutant fragment, which forms amyloid fibrils in the diseased brain of patients with GSS (52, 53) form heterogeneous ion channels (Fig. 2). In contrast, the longer PrP fragments PrP(82–146) SC and PrP(82–146) (106–126) SC, which contain scrambled PrP(106–126) sequence, failed to form definite bona fide ion channels. The PrP(82–146) WT-formed fast cation channels maintain their sensitivity to Cu\(^{2+}\) (Fig. 5), as has been reported previously for PrP(106–126)-formed channels (27, 29). The findings further confirm the significant role of the 106–126 region of PrP in the incorporation and stabilization of aggregated peptide channels that are capable of inducing neurotoxicity (20, 27, 28). Although longer PrP fragments that contain the transmembrane domain sequence 106–126 (Fig. 1) may not prevent ion channel formation, they could affect the distribution of the formed ion channel types.

Recently, more evidence for other channel-forming amyloid peptides has emerged: 1) channel formation by serum amyloid A has been proposed as a potential mechanism for amyloid pathogenesis (17, 59); 2) vesicle permeabilization by protofibrillar \(\alpha\)-synuclein, impli-

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**Fig. 6.** PrP(82–146) WT-formed time-dependent inactivating cation channel. A: control [Cu\(^{2+}\)]\(_{\text{cis}}\). B: 750 \(\mu\)M [Cu\(^{2+}\)]\(_{\text{cis}}\) + 750 \(\mu\)M [Cu\(^{2+}\)]\(_{\text{trans}}\). C: control [RIF]\(_{\text{cis}}\). D: 264 \(\mu\)M [RIF]\(_{\text{cis}}\) + 132 \(\mu\)M [RIF]\(_{\text{trans}}\). Current traces are offset by 120 pA.

**Fig. 7.** Channel formation in the absence and presence of [RIF]\(_{\text{cis}}\). Traces show PrP(82–146) WT fast cation channel formed in control cis solution containing 0 \(\mu\)M RIF (A) and another PrP(82–146) WT fast cation channel (B) formed in a different bilayer from that in A, in the presence of 100 \(\mu\)M [RIF]\(_{\text{cis}}\). Identified current levels are present in long segments of both channels. C denotes the closed state of the channel; O1, O2, O3, and O4 denote the channel opening to current levels of 2.2, 4.4, 6.6, and 8.8 pA, respectively.
cated in the pathogenesis of Parkinson’s disease (58); 3) pore formation by huntingtin protein, implicated in Huntington’s disease (41); and 4) trimeric channel-like structures, implicated in prion neurogenerative diseases (10, 62).

Structural, electrophysiological, and theoretical studies point to the channel diversity of amyloid-forming peptides (see Refs. 6, 10, 27, 31, 32, 37, and 62). Our working hypothesis is that the channel heterogeneity results from different modes of oligomerization of monomers of different PrP intermediates. Although monomers and multimers could be attached to the membrane, multimers are more stable (43). The functioning heterogeneous ion channels indicate the formation of different β-sheet oligomers in the membranes. The β-sheet oligomerization that leads to channel formation represents an early pathway that is different from and precedes that for amyloid fibril formation (see also Ref. 4). The multimeric β-sheet-enriched conformation state of the channel represent a low free energy state in the membrane. The channel is structured such that it catalyzes the binding of other monomers to form comultimeric channels, thus further lowering the energy state of the channel complexes and producing in the membrane structures similar to those seen in the crystal structure studies (62) and also predicted by Chapron et al. (10). The oligomerization-based amyloid and prion channel formation is pH and ion metal sensitive (see Refs. 9, 18, 19, and 33), and the ease of β-sheet oligomerization in the membrane may depend on the pH- and ion metal-sensitive unfolding rate of the unstable monomers.

Several other aggregation-prone peptide intermediates form heterogeneous ion channels with similar biophysical properties that could indicate shared channel structures (23, 26). The presence of different pep-

Fig. 8. Effects of [Cd²⁺]_{cis} on the activity of a PrP(82–146) WT channel, formed in the presence of 100 μM [RIF]_{cis}, activated at V_m between 0 and +140 mV in KCl (250 mM cis/50 mM trans). Current traces are shown for control (0 mM [Cd²⁺]_{cis}) (A), 0.6 mM [Cd²⁺]_{cis} (B), 1.2 mM [Cd²⁺]_{cis} (C), 1.8 mM [Cd²⁺]_{cis} (D), and 2.4 mM [Cd²⁺]_{cis} (E). Current traces are offset by 22 pA.

Fig. 9. Effects of different [Cd²⁺]_{cis} on PrP(82–146) WT-formed cation channel recorded from voltage-clamped optimal bilayers at V_m between −160 and +140 mV in KCl (250 mM cis/50 mM trans). Current-voltage relationships were constructed for the maximal current (I) and mean current (I'), shown in A and B, respectively. Solid lines are fitted to a third-order polynomial for control 0 mM [Cd²⁺]_{cis} (○) and for 0.6 (●), 1.2 (□), 1.8 (●), and 2.4 mM [Cd²⁺]_{cis} (○).

Fig. 10. Dose-dependent effects of [Cd²⁺]_{cis} on the mean current parameter of the PrP(82–146) WT-formed channel. The channel is activated at voltages between +20 and +140 mV in KCl (250 mM cis/50 mM trans). Solid lines are fitted to a third-order polynomial for +20 (○), +40 (●), +60 (□), +80 (●), +100 (○), +120 (●), and +140 mV (▲).
tide conformations that are indicated from the formation of heterogeneous cytotoxic channels complicates the therapeutic approaches of PrP diseases. The formation of heterogeneous ion channels may contribute to enhanced toxicity of aggregation-prone peptides and intermediates. On the basis of the channel heterogeneity reported here, the enhanced toxicity of miniprion (7) could be due to the formation of yet more heterogeneous channels and/or changes in the distribution of channel type, e.g., enhancement in formation of those channels that have large conductances and slow channel kinetics (Fig. 2D). These properties allow such PrP channels to compromise cell regulation very rapidly.

Significance of longer fragments. In essence, prion diseases are membrane linked (see Refs. 12, 51, and 52), and hence the elucidation of the interaction of PrP with cellular membranes is fundamental to an understanding of the molecular mechanism of PrP
tide action. Recent evidence provides further proof of the strong linkage between abnormal accumulation and binding of prion to membranes and neurodegeneration (see Ref. 35). Additionally, data obtained from human brain provide confirm a linkage between a mutation-induced accumulation of a transmembrane form of the prion protein, termed C13PrP, and GSS (16). The use of synthesized segments of PrP mutants linked to GSS and synthetic lipid bilayer membranes has eliminated ambiguities and allowed the assessment of the interaction of PrP mutants with lipid membranes without interference of other cytosolic factors or membrane proteins. The fact that the long PrP(82–146) WT formed heterogeneous channels similar to those formed with PrP(106–126) argues against the possibility that the short channel-forming PrP(106–126) fragment may adopt structural channel conformations that are different from those of the entire PrP mutant. The ability of PrP(82–146) WT to form β-sheet-based channels is in agreement with the properties of the miniprion protein PrP106, which has a high propensity to adopt a secondary structure with high β-sheet content (7), favoring channel formation. In this study, we used PrP(82–146), which is part of a longer PrP fragment (PrP106) also known as “miniprion,” a construct of 106 amino acids (Fig. 1) that was found to sustain PrP replication when expressed in transgenic mice with a PrP knockout genetic background (see Refs. 49 and 50).

PrP106 is derived from mouse PrP and contains two deletions, in the regions 23–88 and 141–176 (39). PrP106 has high β-sheet content, resistance to limited digestion by PK, and high thermodynamic stability, properties that are similar to those of PrPsc106 extracted from scrapie-infected PrP106 transgenic mice (3). In conclusion, the longer PrP fragments that contain the WT region of 106–126 maintained the ability to interact with lipid bilayer membranes and form ion channels that could be part of the cytotoxic mechanism of this GSS-linked mutant prion fragment. Additionally, the channel-forming properties of PrP(82–146) WT validate the findings obtained using the synthetic PrP(106–126) and its usefulness as a tool in PrP channel structure and function investigations.

Rifampicin. RIF has been found to have significant effect on the activity of amyloid peptide (15, 45–56). The findings in Figs. 6–8 suggest that RIF affects intermediates of the channel protein before the assembly of β-sheet channels. The findings indicate that RIF affects the accumulation of PrP(82–146) WT in the lipid bilayer and channel formation. The accumulated PrP(82–146) WT in the bilayer results in fast cation channels that are electrically semisilent or transiently activated (Fig. 8A). In agreement with these findings, several reports have indicated that RIF affects amyloid function, causing the inhibition of peptide aggregation or prevention of amyloid-cell interaction, which leads to protection of cells from amyloid toxicity. The mechanism of RIF-induced inhibition of the toxicity of pre-aggregated amyloid peptides involves binding to peptide fibrils and preventing amyloid-cell interaction (54–56). Similarly, Harron et al. (15) found that RIF can arrest the membrane activity of human islet amyloid polypeptide independently of amyloid formation. Other compounds, e.g., nordihydroguaiaretic acid and tetracycline, have also been found to potently break down preformed Alzheimer’s β-amyloid fibrils in vitro (40).

The fact that the time of RIF application affected the PrP(82–146) WT channel formation but not the channel per se may suggest that RIF action is mediated via certain amyloid configurations. Our data suggest that proto PrP(82–146) WT channel conformations needed for the formation of the fast cation channels are RIF sensitive (Fig. 7B) and that others underlying the formation of large conductance time-dependent inactivating channels are RIF insensitive (data not shown). Our data also suggest that RIF does not fully inhibit PrP(82–146) WT interaction with bilayers, although it affects the properties of PrP(82–146) WT fast cation channels formed in its presence. Furthermore, RIF cannot prevent the Cd
to-stimulated PrP(82–146) WT accumulation of the fast cation channel subunits or block the large-conductance PrP(82–146) WT channel. The findings obtained in this study using PrP(82–146) indicate that it is not unreasonable to propose that PrPsc has the conformational ability to interact with cellular membranes and form heterogeneous ion channels. We suggest that the formation of some of these channels could be slowed with RIF. Furthermore, we propose that the pharmacological approach that involves preventing assembly and incorporation of channel-forming amyloid intermediates into membranes could be a common therapeutic strategy for amyloid-linked pathologies.

Effects of Cd
to on PrP(82–146) fast cation channels. The channel types formed with PrP(82–146) differ in their regulation. Unlike the PrP(82–146) WT fast cation channels, the large-conductance inactivating channels could not be modulated with [Cu
to]lis, [Cd
to]lis, or [RIF]lis. The effects of Cd
to on PrP(82–146) WT include 1) an increase in channel transition between four levels, 2) no change in current amplitude, and 3) lack of complete single-current transitions to levels larger than the full current transition of a single-channel
point to a steady unified channel conductance in the presence of Cd$^{2+}$. Therefore, the Cd$^{2+}$-induced increase in current transitions are the consequences of the enhancement in accumulation of PrP(82–146) WT in the bilayer and increase in single-channel activity. The effects of Cd$^{2+}$-induced accumulation of PrP(82–146) WT in the bilayer and channel formation were concentration dependent (Figs. 8, 9B, and 10). Changes in the homeostasis of the brain’s transition metals have been linked to neurodegenerative diseases (9). The Cd$^{2+}$ concentration of 0.6 mM at which enhanced channel activity was observed (Fig. 8) is higher than that in the brain. Higher Cd$^{2+}$ concentrations in our study shortened the experiment’s time course for Cd$^{2+}$-induced PrP(82–146) accumulation and channel formation in bilayers. This approach allowed us to observe and study these channels. On the other hand, in a diseased brain the time course is longer, and therefore small changes in the concentration of transitional metals will lead, over time, to enhanced accumulation of PrP mutant and ion channel formation in membranes. Cd$^{2+}$, Cu$^{2+}$, and Zn$^{2+}$ are also known to modulate ion channels, including those formed in bilayers and in excised neuronal membrane patches (44) and in tetracarcinoma cells (46). The data presented in this study suggest that Cd$^{2+}$ binds to synthetic PrP(82–146) WT and also to PrP(82–146) (127–146) SC, peptides enhancing the assembly of these peptides into fast cation channels and hence the cationic current (Figs. 8–10). This cation flow through the channel could account for changes in Ca$^{2+}$ and electrolyte homeostasis associated with prion diseases.

We thank Dr. W. L. F. Armarego and R. McCrat for suggestions and critical reading of the manuscript. The laboratory assistance of K. N. Laadchahi is greatly appreciated.

DISCLOSURES

This research work is supported by National Health and Medical Research Council Project Grants 970122 and 129080 and Australian Research Council Small Research Grants F0047 and F01008 (to J. I. Kourie) and by Italian Ministry of Health Grant RF 2001.96, Italian Ministry of University and Research Grant PRIN 2001, and European Union Grant QLRT 2001-00283.

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