Altered toxicity of the prion protein peptide PrP<sub>106–126</sub> carrying the Ala<sup>117</sup> → Val mutation

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The inherited prion diseases such as Gerstmann–Straussler–Scheinker syndrome (GSS) and Fatal Familial Insomnia and some forms of Creutzfeldt–Jakob Disease (CJD) are linked to distinct point mutations within the gene coding for the cellular isoform of the prion protein (PrP<sup>c</sup>). The point mutations in the human PRNP gene include the codons 102, 117, 145, 198, 217 (all GSS) as well as 178, 200 and 210 (mostly CJD) [4, 5]. In CJD or GSS patients PrP<sup>c</sup> accumulates as the abnormal scrapie isoform of the prion protein (PrP<sup>Sc</sup>). Patients with GSS are normally diagnosed with the disease in the fifth decade of life, the first signs of disease usually being motor disturbances. The form of prion disease in GSS is characterized by multicentric amyloid plaques in cerebellum and cortex. The extent and nature of neuropathological changes and clinical signs is extremely variable even when patients have the same mutation, and also with patients from the one family. Such variability suggests that prion ‘strains’ associated with artificial inoculation of mice have little relevance to naturally occurring prion diseases.

Some of the mutations associated with GSS occur only in a small number of families. Whereas GSS with the P102L (Pro<sup>102</sup> → Leu) mutation occurs in greater than 30 known families, the A117V (Ala<sup>117</sup> → Val) mutation occurs in only three [6–8]. The A117V mutation requires two changes in the genetic code to produce the amino acid change, possibly suggestive of a rare occurrence. Some forms of GSS are associated with neuropathology inclusive of neurofibrillary tangles formed from abnormally deposited tau protein. Although this is mostly associated with the F198S (Phe<sup>198</sup> → Ser) [9] and Q217R (Gln<sup>217</sup> → Arg) mutations it is also known to occur in one case with A117V [10].

The A117V mutation lies within a region of the protein conserved in every known mammalian sequence of the prion protein (PrP) [11]. The high conservation of this region suggests that it is important that no mutation be introduced into the function or to prevent pathological consequences. Previous detailed analysis of peptides spanning this region (PrP<sub>106–126</sub>) allows analysis of the effect of this mutation on this peptide.

PrP<sub>106–126</sub> applied to cultured cells has been used as a model for the neurotoxicity of PrP<sup>Sc</sup>. In culture both PrP<sup>Sc</sup> [12] and PrP<sub>106–126</sub> [13, 14] require neuronal expression of PrP<sup>Sc</sup> and the presence of activated microglia in order to exert a toxic effect. PrP<sub>106–126</sub> causes a reduction in the resistance of neuronal cells to oxidative stress [15], possibly through inhibiting incorporation of copper into superoxide dismutase [16] and possibly other enzymes. PrP<sub>106–126</sub> also inhibits astrocytic glutamate uptake [17], which may have consequences for neuronal survival in the latter stages of prion disease [18] as a result of the induction of astrocyte proliferation [19].

In this study it was shown that synthesizing PrP<sub>106–126</sub> with the substitution of valine for alanine at amino acid residue 117 altered the peptide making it more neurotoxic, altering its β-sheet content and enhancing its ability to inhibit tau-stimulated microtubule assembly. This study suggests some possibilities as to how the A117V mutation can result in a pathological phenotype.

METHODS

Unless stated, pharmacological agents were purchased from Sigma. Calciseptine was from Latoxan (Rosans, France).

Abbreviations used: CJD, Creutzfeldt–Jakob Disease; GSS, Gerstmann–Straussler–Scheinker syndrome; LLME, L-leucine methyl ester; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide; PrP, prion protein; PrP<sup>Sc</sup>, scrapie isoform of PrP; PrP<sup>c</sup>, cellular isoform of PrP; PrP<sub>106–126</sub>, wild-type sequence of PrP<sub>106–126</sub>; PrP<sub>106–126(117V)</sub>, PrP<sub>106–126</sub> carrying a valine substitution at position 117; Pr<sup>Sc</sup>, PrP knockout mice.

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Animals

The PrP knockout mice (Prnp<sup>−/−</sup>) used in this study were those generated by Bül et al. [20]. The wild-type mice used were descendants of an F1-generation mouse produced by interbreeding the original parental strains [C57BL/6J and 129/SvEv] mice used to generate the Prnp<sup>−/−</sup> mice.

Neuronal cell culture

Preparation of cerebellar cells from 6-day-old mice (P6) was as described previously [14]. Briefly, the cerebella were dissociated in Hanks medium (Gibco) containing 0.5 % trypsin (Sigma) and plated at 1–2 × 10<sup>6</sup> cells/cm<sup>2</sup> in 24-well trays (Falcon) coated with poly-d-lysine (50 μg/ml, Sigma). Cultures were maintained in Dulbecco’s minimal essential media (Gibco) supplemented with 10 % (v/v) foetal calf serum, 2 mM glutamine and 1 % (v/v) antibiotics (penicillin, streptomycin, fungizone; Gibco). Cultures were maintained at 37 °C with 6 % CO<sub>2</sub> for 10 days. Peptides were added to cultures initially and on day three. The peptide concentration was maintained at 80 μM. Cell survival was determined on day seven. 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide (MTT; Sigma) was diluted to 200 μM in Hanks solution and added to the cultures for 2 h at 37 °C. The MTT formazan product was released from the cells by the addition of DMSO (Sigma) and measured at 570 nm in a Unicam UV2 spectrophotometer (ATI Unicam). Relative survival in comparison with untreated control could then be determined.

For co-culture experiments, cerebellar cells were plated as normal in 24-well trays. Microglia were plated in tissue-culture inserts (Falcon) with 0.4 μm pores. Peptides could then be applied directly to the lower wells and to the insert. After peptide treatments, the inserts were removed and MTT assays carried out on the cerebellar cells as described above.

Glia cell culture

Microglia were isolated as described previously [19]. Briefly, cortices from newborn mice were dissociated with trypsin and seeded into tissue-culture flasks (Falcon). When cultures were confluent, microglia were isolated by rapid shaking for 2 h. The floating cells were collected and plated for 30 min. The non-adhering cells were discarded. The adhering cells were identified as pure cultures of microglia by immunostaining for ferritin as described previously [19].

Peptides

Peptides used in these experiments included PrP<sub>106–126</sub>(117A) with the sequence KTNMKHAMAGAAVGGGLG, derived from amino acid residues 106–126 of the human PrP sequence, and the mutant version of the peptide, PrP<sub>106–126</sub>(117V), based on the human form of the sequence found in GSS patients, KTNMKHAMAGAVGAVGGGLG. Peptides were produced in house by the Protein and Nucleic Acid Chemistry facility or produced as described previously [14].

Analysis of fibril formation

The ability of PrP peptides to form fibrils was checked by negative staining with phosphotungstic acid and transmission electron microscopy. Briefly, a droplet of 10 μM peptide was incubated on a formvar-coated electron microscopy grid for 2 min. Excess solution was removed and the grid allowed to air dry. Analysis of triplicate preparations of the peptides was performed on a JOEL transmission electron microscope at 80 kV.

CD analysis

CD spectra were recorded for PrP peptides in a CD6 spectropolarimeter (Jobin Yvon, Division d’Instrumente S.A.), calibrated with ammonium d-camphor-10-sulphonate by a method similar to that described previously [20a]. Peptide samples (2 mg/ml) prepared in 10 mM sodium phosphate (pH 7.4) were measured in cuvettes with a 0.1 mm pathlength. The spectrum from 195–250 nm was analysed with a step resolution of 0.5 nm at a temperature of 23 °C. Five scans were averaged and the background from buffer was subtracted. Spectra are presented as molar ellipticity [θ]<sub>220</sub>.

Tubulin polymerization assays

Two assays were used to assess tubulin polymerization. The first was a tau-independent assay. This assay was as described previously [21]. Tubulin (Sigma) was added to the reaction at 0.5 mg/ml (55 nM). The assay ran for 45 min while measuring the turbidity at 350 nm in a spectrophotometer. The second assay was a tau-dependent assay. This assay was similar to one described by Yoshi and Ihara [22]. The polymerization buffer contained 100 mM Mes (pH 6.5), 1 mM EGTA, 0.5 mM MgCl<sub>2</sub>, 1 mM dithiothreitol, 1 mM GTP and 0.1 mg/ml tau (ICN). The assay was started by the addition of 1.0 mg/ml tubulin (110 nM). The assay ran for 45 min while measuring the turbidity at 350 nm in a spectrophotometer.

Measurements of intracellular Ca<sup>2+</sup>

Cerebellar cell cultures were loaded with fura-2/AM (Molecular Probes) to assess changes in intracellular calcium levels as described previously [23]. Cells were examined after 24 h treatment with PrP<sub>106–126</sub> peptides using epifluorescence stimulation and the level of intracellular calcium determined as described previously [23]. For the assessment of [Ca<sup>2+</sup>]<sub>i</sub>, fluorescence signals were collected from a 15 μm diameter area. This window was centred on the cell soma.

RESULTS

Fibril formation

The ability of the two forms of PrP<sub>106–126</sub> to form fibrils was compared. PrP<sub>106–126</sub> was dried on to electron microscopy grids and stained with phosphotungstic acid. The resulting fibrils, as seen via electron microscopy, are shown in Figure 1. There was no apparent differences in the ability of the peptides to form fibrils.

CD analysis

CD analysis of the PrP peptides used in this study was carried out. The results of the analysis appear in Figure 2. Both peptides were analysed in phosphate buffer as in previous investigations [20a]. The peptides were prepared freshly and analysed immediately. Both PrP<sub>106–126</sub>(117A) and PrP<sub>106–126</sub>(117V) showed a major trough at approx. 197 nm consistent with a random-coil structure. The spectra of both peptides also show a trough at 219–224 nm. This trough is indicative of β-sheet content. However, this trough is much greater for PrP<sub>106–126</sub>(117V) suggesting that β-sheet is a predominant characteristic
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of its structure under these conditions. These results suggest a major difference in the secondary structure of PrP106–126(117V) compared with PrP106–126(117A).

Toxicity

A comparison was made between the mechanism of toxicity of PrP106–126(117A) and the mutant peptide, PrP106–126(117V). PrP106–126 is known to be toxic to neurone-rich cultures such as those produced by postnatal mice. Cerebellar cell cultures from 5–6-day-old wild-type mice were prepared and exposed to various concentrations of PrP106–126(117A) and PrP106–126(117V). After 7 days of treatment the survival of the cultures was compared using an MTT assay. As seen in Figure 3A PrP106–126(117V) was far more toxic than PrP106–126(117A). PrP106–126(117V) showed significant levels of toxicity even at 10 μM (Student’s t test, P < 0.05).

The dependence of PrP106–126(117V) toxicity on PrP<sup>C</sup> expression was investigated by application of the peptides to cerebellar cells derived from mice deficient in PrP<sup>C</sup> expression (Prnp<sup>−/−</sup>). At concentrations of 20 μM and above PrP106–126(117V) was toxic to Prnp<sup>−/−</sup> cerebellar cells while PrP106–126(117A) was not (Figure 3B). This suggests that the toxicity of PrP106–126(117V) was partly independent of PrP<sup>C</sup> expression.

The dependence of PrP106–126(117V) toxicity on the presence of microglia in the cerebellar cell cultures was also examined. Wild-type cerebellar cells were treated with 50 μM l-leucine methyl ester (LLME), an agent that is selectively toxic to
microglia (Figure 4A). Although the toxicity of PrP106–126(117V) was greatly reduced by this treatment (Figure 4A) it was not abolished. In comparison, the toxicity of PrP106–126(117A) was abolished. Addition of purified microglia in coculture with LLME-treated wild-type cerebellar cells enhanced the toxicity of both peptides. Open bars, control; grey bars, LLME treated; black bars, LLME treated and microglia co-cultured. (B) Prnp<sup>+/−</sup> cerebellar cells were co-cultured with an increasing number of wild-type microglia. PrP106–126 (117V) was applied at 80 µM to Prnp<sup>+/−</sup> cerebellar cells with and without microglial co-culture. An MTT assay was carried out after 7 days treatment to determine relative survival. Addition of microglia did not enhance the toxicity of this peptide. Values shown are the means ± S.E.M. for a minimum of four experiments.

Figure 4 The requirement of microglia for PrP106–126 toxicity

(A) LLME was applied to wild-type cerebellar cells at 50 µM for 2 h before treatment with 80 µM PrP106–126 peptides. Some LLME-treated cultures were co-cultured with microglia (10<sup>4</sup> cells/well) applied in inserts. After 7 days treatment an MTT assay was carried out on the cerebellar cells only. LLME treatment blocked the toxicity of PrP106–126(117A) but only partially inhibited the toxicity of PrP106–126(117V). Addition of microglia increased the toxicity of both peptides. Open bars, control; grey bars, LLME treated; black bars, LLME treated and microglia co-cultured. (B) Prnp<sup>+/−</sup> cerebellar cells were co-cultured with an increasing number of wild-type microglia. PrP106–126 (117V) was applied at 80 µM to Prnp<sup>+/−</sup> cerebellar cells with and without microglial co-culture. An MTT assay was carried out after 7 days treatment to determine relative survival. Addition of microglia did not enhance the toxicity of this peptide. Values shown are the means ± S.E.M. for a minimum of four experiments.

Inhibition of tubulin polymerization

A previous report has suggested that PrP106–126 can bind to microtubules [24]. The ability of PrP106–126 to inhibit tubulin polymerization in a cell-free system was investigated. Both tau-dependent and tau-independent polymerization of tubulin was assayed using a spectrophotometric assay of turbidity. The presence of 50 µg/ml tau caused rapid polymerization of tubulin (Figure 6A) over a 45 min period, as indicated by increased turbidity. The presence of 10 µg/ml PrP106–126(117A) in this reaction inhibited the reaction slightly but significantly ($P < 0.05$). However, addition of 10 µg/ml PrP106–126(117V) inhibited the reaction much more and the effect was significantly different to that of PrP106–126(117A) ($P < 0.05$; Figure 6A). The final absorbance readings in this assay were reduced, suggesting that the peptide inhibited the achievable level of polymerized tubulin and not necessarily the rate of polymerization.

A different assay of tubulin polymerization not dependent on tau was also used (Figure 6B). In this assay neither peptide inhibited the polymerization of tubulin suggesting that PrP106–126(117V) did not prevent tubulin polymerization. The effect of the peptides in the tau-dependent assay was re-assessed by examining the effects of the peptides on the rate of the reaction. According to Figure 6(A) the reaction was linear within the first 10 min. Therefore the change in absorbance over the initial 10 min was assessed in the presence of 0.1 mg/ml tau and increasing concentrations of peptide (Figure 6C). Both peptides significantly ($P < 0.05$) decreased the rate of tubulin polymerization having a maximal effect at 1 µg/ml. PrP106–126(117V) had a much greater effect than PrP106–126(117A). With concentrations above 1 µg/ml there was a slightly decreased effect possibly due to aggregation of the peptide. Using 1 µg/ml peptide the effect of tau on the rate of tubulin polymerization in the tau-dependent assay was assessed (Figure 6D). As expected tau on its own greatly enhanced the rate of tubulin polymerization in a dose-dependent manner. This effect was diminished by the presence of peptide and especially that of PrP106–126(117V).
Cerebellar cells were treated with 100 μM PrP106–126(117V) for 7 days in combination with a variety of agents at increasing concentrations. Relative survival was measured using an MTT assay. (A) N-Acetylcysteine did not inhibit the toxicity of PrP106–126(117V); (B) MK801 did not inhibit the toxicity of PrP106–126(117V); (C) Verapamil (●) and calciseptine (○) both inhibited the toxicity of PrP106–126(117V); (D) Intracellular calcium concentrations, [Ca^{2+}]_i, of Prnp^o/o cerebellar neurones with and without peptide treatment for 24 h, as determined by imaging using the dye fura-2/AM. PrP106–126(117V) greatly increases the intracellular calcium concentration. Values shown are the means ± S.E.M. for a minimum of four experiments.

These results suggest that PrP106–126(117V) inhibited tau-dependent polymerization of tubulin by preventing the interaction of tau with tubulin.

Further experiments were carried out to test whether destabilization of the cytoskeleton was implicated in the toxicity of these peptides. Two stabilizers were tested, taxol, which stabilizes microtubules, and phalloidin, which stabilizes actin filaments. Increasing concentrations of taxol inhibited the toxicity of 20 μM PrP106–126(117V) but did not inhibit the toxicity of 80 μM PrP106–126(117A) (Figure 7A). Furthermore taxol blocked the toxicity of PrP106–126(117V) completely on Prnp^o/o cerebellar cells (Figure 7B). Phalloidin had no effect on the toxicity of either peptide (Figure 7C). These results suggest that the mechanism of toxicity of PrP106–126(117V) involves destabilization of microtubules causing increased calcium entry via L-type calcium channels.

**DISCUSSION**

This study examined the effect of a known human point-mutation in the PrP gene on the neurotoxicity *in vitro* of the PrP peptide, PrP106–126. The A117V point mutation of GSS probably arose through two distinct point mutations occurring within the one codon (GCA changing to GTG) [4]. However, this is the only known mutation within the sequence of PrP106–126. This region is very highly conserved between mammalian species [11] and also between birds and mammals [25]. This would suggest that any mutation within this region may have extremely deleterious consequences. Patients carrying this mutation may not present with symptoms until late in life implying that the mutation can lie silent until after reproductive life is past. This might not be the case for any other mutation in this region.

This single change in the amino acid sequence of PrP106–126 altered the toxicity of the peptide greatly. The toxicity of PrP106–126 without the mutation has been studied in detail [13–15,18]. In cerebellar cell cultures, this neurotoxicity requires neuronal expression of PrP^C and microglial release of reactive oxygen species. PrP^sc (murine) applied to the same culture system has the same basic neurotoxic mechanism [12]. In comparison PrP106–126 carrying the A117V mutation does not have this mechanism of toxicity.

The mechanism of neurotoxicity of PrP106–126(117V) showed two components. The first component of the neurotoxicity was the same as that of PrP^sc and requires the presence of microglia and neuronal PrP^C expression for toxicity to neurones in cerebellar cell cultures. However, the second component was novel and did not require neuronal PrP^C expression. Furthermore, this component of the toxicity was not enhanced by the presence of extra microglia. This second component could be inhibited by L-type calcium-channel blockers and taxol. The finding that this peptide can inhibit microtubule polymerization suggests that the second element of the toxic mechanism involves destabilization of the cytoskeleton leading to cell death via calcium entry. The difference in the toxic mechanism of the mutant peptide is supported by the finding that the PrP106–126(117V) has a different secondary structure to that of the wild-type peptide.

The role of calcium influx through L-type calcium channels has been suggested for PrP106–126 from previous experiments [23,26,27]. PrP106–126 effects the kinetics of calcium entry into synaptosomes [26], neurones [27] and astrocytes [27], all of which...
can be inhibited by L-type calcium-channel blockers. However, PrP106–126(117A) is not toxic to neurones lacking expression of PrP [13,14] and, as reported previously [23], does not cause an increase in intracellular calcium in PrP- deficient cells. PrP106–126(117V), in contrast, does not require PrP expression to have a toxic effect. Nevertheless, similar to the effect of PrP106–126 on wild-type cells, the toxicity of PrP106–126(117V) can be inhibited by blockers of L-type calcium channels and application of the peptide caused an increase in intracellular calcium. Changes in microtubule stability have been shown to cause changes in calcium entry via L-type channels [28]. Therefore as PrP106–126 can bind to microtubules [24] it is possible that the changes in intracellular calcium measured here are a result of PrP106–126(117V) binding to microtubules.

The peptide PrP106–126 has many similarities to PrPSc in that it is protease resistant, forms fibrils, is neurotoxic and has relatively high $\beta$-sheet content. However, the finding that a modification of PrP106–126 alters the toxic mechanism in vitro suggests that there could be heterogeneity in the mechanism of neurotoxicity of PrPSc. The mechanism of toxicity of any ‘type’ of PrPSc would then depend on the sequence of the protein. Clearly patients from families with different inherited mutations in the PrP gene have quite distinct pathologies [4]. However, this issue is confused by there being considerable variability in pathology among patients carrying the same mutation. However, if there are distinct ‘types’ of PrPSc with different toxic mechanisms then this has broad implications for any treatment of the disease(s).

One important distinction of some forms of GSS that has emerged in recent years is the finding that neurofibrillary tangles are associated with some of the point mutations, especially Y145stop [29], F189S [30] and Q217R [30]. Some cases of A117V also show tangles [10]. It has recently been shown that PrP106–126 can bind tubulin [24] in a cell-free assay. Additionally analysis of cellular uptake of biotinylated PrP106–126 with electron microscopy has confirmed that PrP106–126 can enter the cytosol and bind to microtubules in neurones when applied to cultured cerebellar cells [31]. In the current article PrP106–126(117V) inhibited tau-mediated tubulin polymerization. However, the peptide did not inhibit spontaneous polymerization. This implies that microtubules may form in cells but that tau might be displaced. An accumulation of tau in cells may then result in the formation of paired helical filaments and thus tangles. This intriguing possibility might explain the occurrence of tangles in this form of GSS. However the identification PrPSc binding to microtubules in the cells of GSS patients would be necessary before this could be confirmed.

In summary, a neurotoxic PrP peptide carrying an amino acid substitution mimicking the A117V mutation of GSS has a different toxic mechanism and secondary structure to that of the peptide with the wild-type sequence. This peptide causes destabilization of cytoskeleton by inhibiting tau-dependent tubulin polymerization without the addition of tau was slow (□). Polymerization was accelerated by the presence of the microtubule-binding tau protein (●). PrP106–126(117A) inhibited this reaction slightly (▲), but PrP106–126(117V) inhibited this reaction greatly (■). (B) Tau-independent assay. In the absence of tau neither PrP106–126(117V) (■) nor PrP106–126(117A) (▲) inhibited tubulin polymerization and the reaction occurred at the same rate as it did without the addition of peptide (●). (C) Rate of tubulin formation in the tau-dependent assay in the presence of a set amount of tau (as in A) and increasing concentrations of either PrP106–126(117V) (■) or PrP106–126(117A) (▲). PrP106–126(117V) inhibited the rate of tau-dependent tubulin polymerization in a dose-dependent manner and its effect was greater than PrP106–126(117A). (D) Rate of tubulin formation in the tau-dependent assay in the absence of peptide (E) or with 1 μg/ml PrP106–126(117V) (■) or PrP106–126(117A) (▲). The rate of tubulin polymerization was greatly enhanced by tau in a dose-dependent manner; however, this was inhibited by PrP106–126(117V). Values shown are the means ± S.E.M. for three experiments per time point.

Figure 6 Assays of tubulin polymerization were carried out using spectrophotometric determination of turbidity.

(A) Tau-dependent assay. Polymerization without the addition of tau was slow (□). Polymerization was accelerated by the presence of the microtubule-binding tau protein (●). PrP106–126(117A) inhibited this reaction slightly (▲), but PrP106–126(117V) inhibited this reaction greatly (■). (B) Tau-independent assay. In the absence of tau neither PrP106–126(117V) (■) nor PrP106–126(117A) (▲) inhibited tubulin polymerization and the reaction occurred at the same rate as it did without the addition of peptide (●). (C) Rate of tubulin formation in the tau-dependent assay in the presence of a set amount of tau (as in A) and increasing concentrations of either PrP106–126(117V) (■) or PrP106–126(117A) (▲). PrP106–126(117V) inhibited the rate of tau-dependent tubulin polymerization in a dose-dependent manner and its effect was greater than PrP106–126(117A). (D) Rate of tubulin formation in the tau-dependent assay in the absence of peptide (E) or with 1 μg/ml PrP106–126(117V) (■) or PrP106–126(117A) (▲). The rate of tubulin polymerization was greatly enhanced by tau in a dose-dependent manner; however, this was inhibited by PrP106–126(117V). Values shown are the means ± S.E.M. for three experiments per time point.
polymerization. These results suggest that there may be heterogeneity in the neurotoxicity of PrPSc-dependent on its amino acid sequence.

Figure 7 Effect of cytoskeletal-stabilizing agents on the toxicity of PrP peptides

(A) Wild-type cerebellar cells were exposed to 80 μM PrP106–126(117A) (●) or 20 μM PrP106–126(117V) (○) for 7 days. Taxol treatment inhibited the toxicity of PrP106–126(117V) only, as determined by an MTT assay. (B) Prnp<sup>−/−</sup> cerebellar cells were treated for 7 days with 20 μM PrP106–126(117V). Taxol co-treatment fully blocked the toxicity of this peptide, as determined by an MTT assay. (C) Wild-type cerebellar cells were exposed to 80 μM PrP106–126(117A) (●) or 20 μM PrP106–126(117V) (○) for 7 days and co-treated with phalloidin. An MTT assay after this time indicated that phalloidin did not inhibit the toxicity of either peptide. Values shown are the means ± S.E.M. for a minimum of four experiments.

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