The synthetic peptide PrP-(106–126) has previously been shown to be neurotoxic. Here, for the first time, we report that it induces apoptosis in the human neuroblastoma cell line SH-SY5Y. The earliest detectable apoptotic event in this system is the rapid depolarization of mitochondrial membranes, occurring immediately upon treatment of cells with PrP-(106–126). Subsequent to this, cytochrome c release and caspase activation were observed. Caspase inhibitors demonstrated that while the peptide activates caspases they are not an absolute requirement for apoptosis. Parallel to caspase activation, PrP-(106–126) was also observed to trigger a rise in intracellular calcium through release of mitochondrial calcium stores. This leads to the activation of calpains, another family of proteases. A calpain inhibitor demonstrated that while calpains are activated by the peptide they also are not an absolute requirement for apoptosis. Interestingly a combination of caspase and calpain inhibitors significantly inhibited apoptosis. This illustrates alternative pathways leading to apoptosis via caspases and calpains and that blocking both pathways is required to inhibit apoptosis. These results implicate the mitochondrion as a primary site of action of PrP-(106–126).

Prion-related encephalopathies are a family of neurodegenerative disorders including conditions such as scrapie in sheep, bovine spongiform encephalopathy in cattle, and Creutzfeldt-Jakob disease and Gerstmann-Strausser-Scheinker syndrome among others in humans. They are characterized by vacuolation of the neuropil, neuronal loss, and gliosis (1). In many cases this is also accompanied by the extracellular accumulation of the scrapie isoform (PrPSc) of the normal cellular prion protein (PrPC), which can aggregate into fibrils in the extracellular matrix (2).

PrPSc is widely believed to be the infectious agent of these diseases (3), and the formation of PrPSc is thought to be via a post-translational conformational change by which PrPC complexes with PrPSc to yield two molecules of PrPSc (4). PrPSc is a cell surface protein mainly expressed in the neuronal and glial cells of the central nervous system. The exact function of PrPC remains unknown, although recent studies have implicated it in copper metabolism (5, 6) and signal transduction (7). Its expression is necessary for the pathogenesis of the spongiform encephalopathies (8).

Apoptosis is a physiologically important cellular suicide pathway, which has also been implicated in a number of pathological conditions (9). There is some evidence to indicate that the mechanism of neuronal cell death in prion diseases is apoptosis as apoptotic neurons have been observed in the brain of scrapie-infected sheep (10), the brain and retinas of mice infected with the 79A strain of scrapie (11), and the brain of human Creutzfeldt-Jakob disease patients (12).

A synthetic peptide corresponding to residues 106–126 of human PrP (PrP-(106–126)) has previously been found to induce apoptosis in primary rat hippocampal cultures (13), primary mouse cerebellar cultures (14), the rat pituitary clonal cell line GH3 (15), and more recently in mouse retinae in vivo (16). PrP-(106–126) is one of a number of peptides corresponding to sequences within fragments of amyloid proteins isolated from the brains of patients suffering from Gerstmann-Strausser-Scheinker syndrome (17). It retains the ability of PrPSc to aggregate into amyloid-like fibrils and the tendency to adopt a mostly β-sheet structure (18). Residues 106–126 of PrP constitute a region maintained in all the PrP isoforms that have been found to accumulate in the brains of patients suffering from prion diseases. Under normal physiological conditions a catastrophic pathway also leads to cleavage of this region of the prion protein at residues 110 and 111 (19).

The importance of the 106–126 sequence of the prion protein makes it a useful model for the in vitro study of prion-induced cell death. In this study we determine, for the first time, the effect of PrP-(106–126) in human neuroblastoma cells in vitro and demonstrate its ability to induce apoptosis. Furthermore, we establish the mitochondrion as a target of PrP-(106–126) action and show that the peptide activates two distinct biochemical pathways involving caspases and calpains, which both result in apoptosis.

**EXPERIMENTAL PROCEDURES**

**Peptides and Drugs**—PrP-(106–126) (KTNMKHMAGAAAAGAVVG-GLO) and PrP-(106–126) scrambled (scr) (AVHTGLGAMALNNVVG-GAAGL) were synthesized and purified by MWG Biotech (Milton Keynes, UK). Peptides were dissolved in sterile phosphate-buffered saline (PBS) to a concentration of 1 mM before use and were freshly prepared before each experiment. The inhibitors z-VAD-fmk (Enzyme Systems Products, Livermore, CA) and calpeptin (Sigma) were added to cells 15 min prior to drug/peptide treatments.

**Thioflavin-T Binding Assay**—The degree of peptide aggregation was measured fluorimetrically with thioflavin-T binding (20). Aged prepara-
tions of peptides in PBS were added to 50 mM glycerine, pH 9, with 2 μM thioflavin-T (Sigma) to a concentration of 100 μM. Samples were incubated at room temperature for 5 min, and then fluorescence was measured on a SpectraMax Gemini fluorimeter with excitation and emission maxima of 435 and 485 nm, respectively. Samples were prepared in triplicate.

Cell Culture—The adherent human neuroblastoma cell line SH-SY5Y was cultured in RPMI 1640 medium (Life Technologies, Inc.) supplemented with 10% fetal calf serum (Life Technologies, Inc.), 1% penicillin/streptomycin (Life Technologies, Inc.), and 1% l-glutamine (Life Technologies, Inc.). Cells were maintained at 37 °C in a humidified 5% CO2 atmosphere. For experiments requiring serum-free medium, Cells were transferred to serum-free RPMI 1640 medium supplemented with 1% Growth Medium Supplement-A (Life Technologies, Inc.), 1% penicillin/streptomycin, and 1% l-glutamine. When being passed or harvested for analysis cells were lifted using trypsin/EDTA.

MTT Assay—Cytotoxicity was assessed by the conversion of MTT (Sigma) to a formazan product. After appropriate incubation of cells with peptides, MTT was added to each well to a final concentration of 0.25 mg/ml and then incubated for 4 h at 37 °C. Microtiter plates were then centrifuged at 200 × g for 5 min. The reaction was terminated by removal of the supernatant and addition of 100 μl of Me2SO to each well. Following thorough mixing to dissolve the formazan product, the plates were read at 620 nm on a microELISA plate reader. Assays were performed in triplicate.

Annexin V Binding—Cell viability was assessed by flow cytometry that monitored annexin V binding and propidium iodide (PI) uptake simultaneously. After appropriate incubation with drugs/peptides, cells were resuspended in annexin V binding buffer and then treated with annexin V (1×) and PI (5 μg/ml) for 5 min at room temperature. Samples were then analyzed by fluorescence on a FACScan flow cytometer (Becton Dickinson, Oxford, UK). Fluorescence was measured through a 530/30 band pass filter (FL-1) to monitor annexin V binding and through a 585/42 band pass filter (FL-2) to monitor PI uptake. An increase in FL-1 fluorescence is indicative of apoptosis before an increase in FL-2, indicative of secondary necrosis, is observed.

DNA Fragmentation Assay—DNA was isolated and electrophoresed on 10% agarose gels by the method of McGahan et al. Western Blotting—Cells were lysed in RIPA buffer (50 mM Tris-HCl (pH 7.4), 1% Nonidet P-40, 0.25% sodium deoxycholate, 1 mM EGTA, 1 mM Na3VO4, 1 mM NaF, 150 μM NaCl, 0.1% phenylmethylsulfonyl fluoride, and 1 μl of protein inhibitor mixture (1 μg/ml antipain, 1 μg/ml aprotinin, 1 μg/ml chymostatin, 0.1 μg/ml leupeptin, and 1 μg/ml pepstatin)) and put on ice for 40 min. Samples were then centrifuged at 20,000 × g for 30 min. The supernatant was transferred to Eppendorf tubes, and protein concentrations were determined using a Bio-Rad protein assay reagent. Protein was separated by SDS-polyacrylamide gel electrophoresis (15% gel, 30 μg of protein/sample) and then transferred to a nitrocellulose membrane. Proteins were detected using primary antibodies and the ECL detection reagent (Amer sham Pharmacia Biotech). The antibodies used were mouse monoclonal anti-cytochrome c (Dako, Cambridge, UK), rabbit monoclonal anti-Bcl-Xs (Calbiochem), rabbit monoclonal anti-Bax (DAKO, Cambridge, UK), monoclonal anti-cytochrome c (DAKO), and monoclonal anti-cytochrome c oxidase (DAKO).

Measurement of Mitochondrial Membrane Potential (ψm) —Cells were harvested and treated with 10 μM JC-1 (Molecular Probes) for 15 min at 37 °C (22). Mitochondrial membrane potential was then measured by fluorescence emission on a FACScan flow cytometer (Becton Dickinson). Fluorescence emission was collected through FL-1 on a log scale, and an increase in FL-1 fluorescence is indicative of an increase in calcium levels.

Isolation of Mitochondria and Cytochrome c Detection—Cells were harvested, washed in 1 ml of homogenizing medium (1 mM sucrose, 1 mM Tris-HCl (pH 7.4), 50 mM EGTA, and 1% bovine serum albumin), resuspended in 80 μl of homogenizing medium, and Dounce-homoge nized. Mitochondrial fractions were centrifuged at 1000 × g for 5 min at 4 °C, and the supernatant was transferred to a clean Eppendorf tube. The supernatant was centrifuged at 10,000 × g for 5 min at 4 °C. The pellet was then washed in 100 μl of Wash 1 (1 mM sucrose, 1 mM Tris-HCl, 1% bovine serum albumin (pH 7.4), 50 mM EGTA, and 1 mM KCl), centrifuged at 10,000 × g for 5 min at 4 °C, and the supernatant was transferred to a clean Eppendorf tube. The supernatant was centrifuged at 100,000 × g for 30 min. The supernatant was transferred to a 10% agarose gel by the method of McGahan et al. Protein determination assay was performed on both fractions, and an equal amount of protein was loaded into each well of a 96-well plate (ideally 100 μg of protein/well). To each well, 32 μl of fluorescent substrate (Suc-Leu-Tyr (AFL-117) (Enzyme Systems Products)) was added, and the total volume of each well was brought to 200 μl with imidazole buffer (100 mM imidazole, 5 mM MgCl2, 1 mM mercaptoethanol, 10 mM CaCl2, 4% Me2SO in H2O). Samples were incubated at 37 °C for 30 min after the addition of the substrate. Fluorescence was then measured on a fluorometer (Spectra Max Gemini, Molecular Devices) with excitation and emission wavelengths of 400 and 505 nm, respectively.

Glutathione Assay—Cells were washed once in PBS and then resuspended in 650 μl of PBS. Next 3.25 μl of 10 mM monochlorobimane (Molecular Probes) was added to each sample. Samples were measured in triplicate, so 200 μl of each sample was aliquoted into three wells of a 96-well plate. Samples were incubated at room temperature for 15 min in darkness and then read on a fluorometer (Spectra Max Gemini, Molecular Devices) with excitation and emission maxima of 395 and 482 nm, respectively.

Catalase Assay—The activity of catalase was measured by following the decrease in absorbance at 240 nm due to H2O2 decomposition (23). Activity was measured as rate of change of absorbance (μOD/min).

Calcium Measurements—Changes in intracellular calcium concentration were detected by loading cells with FLUO-3 (1×) (Molecular Probes) for 15 min prior to sample collection and measurement of fluorescence on a FACScan flow cytometer. Fluorescence emission was collected through FL-1 on a log scale, and an increase in FL-1 fluorescence is indicative of an increase in calcium levels.

RESULTS

PrP-(106-126) Forms Aggregates and Induces Apoptosis in SH-SY5Y Cells—PrP-(106-126) has previously been reported to induce cell death as a result of its ability to form aggregates (13, 24). The aggregation status of the peptide in this study was ascertained by means of a thioflavin-T assay (Fig. 1A). Thioflavin-T fluorescence increases in the presence of protein aggregates. PrP-(106-126) was seen to aggregate immediately at day 0 when prepared at 100 μM in PBS, and over time it gradually increases its aggregation status. The scrambled version of the peptide shows little aggregation even over time. PrP-(106-126) was observed to induce cell death in a dose-dependent manner over time as measured by MTT assay (Fig. 1B). MTT is converted to a formazan product by mitochondrial enzymes, which become inactive as the cell dies. Measurement of this formazan product is an indicator of cell viability. The mechanism of cell death induced by PrP-(106-126) was shown to be apoptosis by annexin V binding (Fig. 1C). Annexin V binds to phosphatidylserine, which flips from the inner to the outer leaflet of the cell membrane during apoptosis (73). Apoptosis could be detected as early as 2 h after treatment when 22% of the cell population were annexin-positive. Cells were incubated with annexin V and PI simultaneously The population of cells in the lower left quadrant represents viable cells (Fig. 1C). An increase in FL-1 represents annexin V-positive cells in the
lower right quadrant. This is the apoptotic population. The final shift in FL-2 up to the top right quadrant represents PI-positive cells, indicative of membrane permeability and secondary necrosis. The percentage of the overall population in each quadrant is given in the circles. D, DNA ladder patterns confirm that death induced by PrP-(106–126) is apoptotic in nature. Lane A, untreated; lane B, 0.5 μM staurosporine as positive control; lane C, 100 μM PrP-(106–126); 24 h; lane D, 100 μM PrP-(106–126). 48 h; lane E, 100 μM PrP-(106–126)scr.

**Figure 1.** Peptide aggregation status and its induction of apoptosis in the SH-SY5Y cell line. A, PrP-(106–126) was found to aggregate immediately when prepared in PBS at a concentration of 100 μM as measured by Thioflavin-T fluorescence. Its aggregation was observed to increase slowly over 8 days. PrP-(106–126)scr showed a much lesser degree of aggregation that remains constant over time. Results are expressed as the mean ± S.E. B, PrP-(106–126) was seen to induce death in the SH-SH5Y cell line in a dose-dependent manner as measured by the MTT assay at 24 h. PrP-(106–126)scr (○) induced no death. Results are expressed as the mean ± S.E. C, 100 μM PrP-(106–126) induces apoptosis within 2 h as compared with controls, which were untreated or treated with 100 μM PrP-(106–126)scr. Apoptosis was measured by annexin V binding via flow cytometry (represented by an increase in FL-1). Secondary necrosis is indicated by a subsequent increase in FL-2. The percentage of the overall population in each quadrant is given in the circles. D, DNA ladder patterns confirm that death induced by PrP-(106–126) is apoptotic in nature. Lane A, untreated; lane B, 0.5 μM staurosporine as positive control; lane C, 100 μM PrP-(106–126); 24 h; lane D, 100 μM PrP-(106–126), 48 h; lane E, 100 μM PrP-(106–126)scr.
change in their Bcl-2 levels. A concentration of 100 µM z-VAD was shown to block caspase-3 activity as measured by anti-active caspase-3 antibody on the FACScan flow cytometer (Fig. 3C). Thus, the inhibition of caspases maintains the protein levels of the antiapoptotic Bcl-2 even in the presence of PrP-(106–126). The same treatment of cells with z-VAD did not inhibit mitochondrial depolarization (Fig. 3D), placing this event upstream of caspase activation and Bcl-2 degradation. More importantly, z-VAD did not inhibit apoptosis despite blocking caspase-mediated Bcl-2 degradation, indicating that caspases are not necessary for PrP-(106–126) to induce cell death.

Oxidative Stress Was Not Induced by PrP-(106–126)—With caspases found to be nonessential for apoptosis in this system, another mechanism must be at work. Maintaining focus on the mitochondrion, as this was the site of the earliest observed effect of PrP-(106–126), we investigated the possible role of oxidative stress due to disruption of mitochondrial function as this is the primary site of intracellular reactive oxygen species production. Oxidative stress has been shown to have a role in a number of apoptotic systems (30, 31). We examined the intracellular levels of peroxides, superoxide anion, and nitric oxide as well as the activities of Mn-superoxide dismutase and Cu,Zn-superoxide dismutase (data not shown). We also looked at glutathione levels using the fluorescent monochlorobimane probe (Fig. 4A) and the activity of catalase by following the decrease in absorbance at 240 nm due to H₂O₂ decomposition (Fig. 4B) in response to PrP-(106–126). Glutathione not only acts as a reactive oxygen species scavenger but also functions in the regulation of the intracellular redox state, and catalase is the primary defense mechanism against H₂O₂. None of the aforementioned changes appreciably in response to PrP-(106–126). Stauroporine (0.25 µM) and valinomycin (50 nM) were used as positive controls as both these drugs induce oxidative stress in SH-SY5Y cells. The peptide was not found to predispose the cells to death by a secondary oxidative insult either (data not shown).

Calcium Homeostasis Was Altered by PrP-(106–126)—Another function of the mitochondrion is in the regulation of intracellular calcium levels, therefore we investigated whether PrP-(106–126) could be exerting an effect through the deregulation of calcium homeostasis. Using the fluorescent probe FLUO-3 we demonstrated an intracellular rise in calcium levels (represented by an increase in FL-1 fluorescence) in response to PrP-(106–126) (Fig. 5A). The response is immediate, and calcium levels reach a sustained peak by 30 s. This experiment was repeated in the presence of EGTA, a calcium chelator, to show that the source of the calcium was intracellular (data not shown). BAPTA-AM, an intracellular calcium chelator, was found to inhibit this increase in calcium levels, confirming the intracellular nature of the source of the calcium rise (Fig. 5B). The two major calcium stores in the cell are the endoplasmic reticulum and the mitochondria. To determine which was releasing calcium into the cytosol in this system we used thapsigargin, which causes a rapid release of endoplasmic reticulum calcium stores. Upon treatment of cells with thapsigargin, an increase in calcium levels was observed using the FLUO-3 probe. Once that response had reached its maximum, cells were treated with 100 µM PrP-(106–126), and a further increase in calcium was observed (Fig. 5C). This indicates that PrP-(106–126) releases calcium from a site other than the endoplasmic reticulum. The mitochondria make up the only other store of calcium in the cell large enough to account for the PrP-(106–126)-induced rise in calcium levels.

Calpain Activity Increased in Response to PrP-(106–126)—We next examined the activity of calpains in response to the PrP-(106–126)-induced rise in intracellular calcium. The calpains are a group of calcium-activated proteases that have also been implicated in apoptosis. They can exist in active and inactive forms associated with the cell membrane or the cytosol (32–34). Within 5 min of treating cells with 100 µM PrP-(106–126) an increase in calpain activity was evident in both the cells in cytosolic and membrane fractions as measured using a fluorescent calpain substrate (Fig. 6A). Pretreatment of cells with the calpain inhibitor calpeptin was found to inhibit the activity of the calpains induced by PrP-(106–126) but not cell death. However, when cells were pretreated with a combination of 100 µM z-VAD and 100 µM calpeptin, apoptosis induced by 100 µM PrP-(106–126) was significantly inhibited (Fig. 6B).
Assemblies (36). The scrambled version of the peptide, PrP-(106–126)sc, does not show the same tendency to aggregate. This is reflected in our observation that PrP-(106–126) induces apoptosis as measured by the apoptotic hallmarks of phosphatidylserine flipping and DNA cleavage, whereas PrP-(106–126)sc does not.

Mitochondrial dysfunction is a well documented event in apoptosis (37, 38). A process known as permeability transition (PT) appears to be responsible for the loss of $\Delta m$, leading to the opening of the PT pore and release of solutes from the mitochondrion (39–41). Among the proteins released are apoptosis-inducing factor and cytochrome $c$. Release of these proteins leads to activation of caspases, a family of serine threonine proteases, and subsequently apoptosis (42). The present study has shown that PrP-(106–126) induces a decrease in mitochondrial membrane potential, leading to the release of cytochrome $c$ into the cytosol and the activation of caspase-3. Among the caspase targets cleaved in this system is Bcl-2. The Bcl-2 family of proteins encodes both positive (Bax, Bcl-XL, Bad, and Bak) and negative (Bcl-2, Bcl-w, and Bcl-XL) regulators of apoptosis, whose primary site of action appears to be at the mitochondrion. Furthermore, it appears that relative ratios and interactions between family members is a key factor in deciding the fate of a cell (43). Bcl-2 is an antiapoptotic protein capable of inhibiting apoptosis induced by a wide variety of apoptotic stimuli in a broad range of cell types (44). PrP-(106–126) has previously been reported to have an effect on Bcl-2 levels in primary rat cortical cultures (45), and the amyloid-$\beta$ peptide of Alzheimer’s disease, similar in some respects to PrP-(106–126), has been shown to cause a 50% decrease in the level of Bcl-2 protein in primary human neuron cultures within 6 h (46).
Prion Peptide Induces Apoptosis via Mitochondrial Disruption

We observed that Bcl-2 becomes completely degraded in SH-SY5Y cells within 15 min of treatment with PrP-(106–126), whereas the levels of Bax (proapoptotic) and Bcl-XL (antiapoptotic) were not seen to change. This is consistent with the fact that a caspase-3 cleavage site is present in the loop domain of Bcl-2 (29). Furthermore, z-VAD, a pan caspase inhibitor, was found to block the depletion of Bcl-2. Such a cleavage would mean that Bcl-2 is losing its BH4 domain, which is essential to its antiapoptotic activity (47). The resulting 23-kDa C-terminal Bcl-2 fragment has also been shown to have proapoptotic activity (48). We were unable to detect breakdown products of Bcl-2 even when a polyclonal antibody was used (data not shown). This is possibly due to another factor bound to Bcl-2 that is obscuring the epitope sites. PrP has been found to directly associate with the C terminus of Bcl-2 in the yeast two hybrid system (49). Perhaps PrP-(106–126) is also capable of binding to Bcl-2 or of inducing cellular PrP to bind to it.

Despite its inhibition of Bcl-2 degradation, z-VAD was not found to inhibit mitochondrial depolarization or apoptosis. This illustrates that depolarization of the mitochondria occurs upstream of caspase activation and Bcl-2 cleavage, again highlighting the mitochondria as a key target for PrP-(106–126). But this also indicates that although caspases are activated by PrP-(106–126) to assist in apoptosis, it would appear that their activation is not essential for completion of the cell death program. This is not to be unexpected as there is now much evidence for the existence of caspase-independent apoptosis (39, 50–52). A recent study showing that caspase-3 activation by β-amyloid and prion proteins is independent from their neurotoxic effects supports this conclusion (53).

If caspases are not essential to apoptosis in this system, then another factor is capable of mediating the cell death program. We first examined the possible role of oxidative species, which are known to be mediators of apoptosis in a number of systems (30, 31), including some instances of neuronal apoptosis (54). There have been many reports relating oxidative stress to cell death induced by PrP-(106–126) and PrPSC (55–57). However, we found no relationship between cell death induced by PrP-(106–126) and oxidative stress in this cell line.

The mitochondria are also involved in the regulation of calcium homeostasis (58). Recently it has been found that calcium homeostasis is disrupted in cerebellar granule cells of prion-deficient mice (59) and in the hypothalamic gonadotropin-releasing hormone neuronal cell line treated with PrP-(106–126) and β-amyloid (60). PrP-(106–126) has also been found to exert effects on calcium homeostasis through impairment of L-type voltage-sensitive calcium channels (15, 61, 62). Calcium is also a known mediator of apoptosis in response to many stimuli (63). We observed a rapid and sustained increase in the cytosolic concentration of calcium in response to PrP-(106–126). Furthermore, we have eliminated the endoplasmic reticulum as a possible source for this calcium. The only other calcium store in the cell large enough to account for the rise in calcium levels observed is the mitochondria. The calcium chelator BAPTA-AM, which buffered the rise in calcium, did not, however, inhibit mitochondrial membrane depolarization. This could indicate that calcium release is upstream of PT, which leads to mitochondrial depolarization. PT is known to be induced by high intracellular calcium (58). However, it appears more likely that membrane depolarization and calcium release are occurring simultaneously as a result of PT as observed in calcium-treated rat liver and brain suspensions (64). Either way PT is triggered by high calcium concentrations, so an initial release of calcium will serve to further enhance PT, speeding up mitochondrial membrane depolarization and releasing more calcium. This could account for the speed at which both these events reach their peak.

As a result of the raised intracellular calcium, calpains were observed to increase in activity. Calpain I, a neutral calcium-activated protease, has previously been reported to be involved in neuronal apoptosis (65). Calcium also activates the phosphatase activity of a calcineurin-calcmodulin complex, which dephosphorylates Bad, allowing it to translocate to the mitochondria where it plays a role in cytochrome c release (66).
Prion Peptide Induces Apoptosis via Mitochondrial Disruption

Using the calpain inhibitor calpeptin, we attempted to rescue PrP-(106–126)-treated cells from apoptosis. But calpeptin was not sufficient to rescue the cells. However, when used in combination with z-VAD to block both caspase and calpain activity, there was a significant reduction in the levels of apoptosis observed. Hence PrP-(106–126) activates two pathways leading to apoptosis. One is governed by caspases, the other is governed by calpains. We envisage much cross-talk between these pathways under normal circumstances, but when one of the pathways is blocked the other is capable of completing the cell death program alone.

The initiation site of the apoptotic pathway in this system, whether involving caspases or calpains, is at the mitochondrion, and this appears to be the principal target of PrP-(106–126) toxicity. There is evidence in the literature supportive of a role for mitochondrial dysfunction in other neurodegenerative disorders such as Alzheimer’s disease (67) and a form of Parkinson’s disease (68). Studies of scrapie-infected hamsters have reported physical disruption of the mitochondria as well as decreased activity of important mitochondrial enzymes (69). PrP-(106–126) has been shown to be capable of uptake into cells in vitro (70), and just how this event occurs is currently under study. After uptake into the cell, the peptide may act directly on the mitochondria. One possible site of interaction is with Bcl-2, which is primarily located on the mitochondria. Bcl-2 has been found to bind PrPC in the yeast two-hybrid system (49). Evidence also exists for possible functional interactions between Bcl-2 and PrPβC; as in PrP-null cells, which are vulnerable to apoptosis induced by serum withdrawal, Bcl-2 can protect against cell death (75). Perhaps an interaction between PrPβ-(106–126) and Bcl-2 interrupts an ant apoptotic activity of Bcl-2 and/or its interaction with native PrPβC. PrPβ-(106–126) has also been found to form ion-permeable channels in planar lipid bilayer membranes (71). These channels are large enough and nonselective enough to mediate the discharge of membrane potential and allow passage of calcium ions. If PrPβ-(106–126) formed channels in the mitochondria, it could cause the observed mitochondrial membrane depolarization and calcium efflux leading to cell death. A similar disruption of calcium homeostasis through channel formation has been reported for Alzheimer’s β-amyloid peptide (72).

In conclusion, we have shown, for the first time, that PrPβ-(106–126) induces apoptosis in a human neuronal cell line. The initial site of action appears to be the mitochondrion where membrane depolarization and calcium release occurs. This is possibly through an interaction with the Bcl-2 protein, which is known to be present on the mitochondrion. Downstream of the mitochondrion the apoptotic program can be completed through the action of either caspases or calpains, and apoptosis can only be blocked by the inhibition of both.

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Fig. 6. Increased calcium levels lead to calpain activation, which can be blocked by calpeptin. A, an increase in activity of the calcium-activated calpain proteases, as measured by a fluorescent calpain substrate, is evident within 15 min of treatment with 100 μM PrP(106–126). Activity was increased in both the cytosolic (■) and membrane (□) fractions. This activity was inhibited using 100 μM calpeptin. Column 1, untreated control; column 2, 100 μM calpeptin; column 3, 100 μM PrP(106–126); column 4, 100 μM PrP(106–126) + 100 μM calpeptin; column 5, 100 μM PrP(106–126) + 100 μM calpeptin. B, calpeptin alone does not stop the cells from undergoing apoptosis, but a combination of z-VAD and calpeptin shows significant inhibition of apoptosis. Column 1, untreated control; column 2, 100 μM PrP(106–126); column 3, 100 μM calpeptin + 100 μM PrP(106–126); column 4, 100 μM z-VAD + 100 μM PrP(106–126); column 5, 100 μM z-VAD + 100 μM calpeptin + 100 μM PrP(106–126).
Prion Protein Fragment PrP-(106–126) Induces Apoptosis via Mitochondrial Disruption in Human Neuronal SH-SY5Y Cells
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doi: 10.1074/jbc.M103894200 originally published online August 30, 2001

Access the most updated version of this article at doi: 10.1074/jbc.M103894200

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