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Involvement of the 5-Lipoxygenase Pathway in the Neurotoxicity of the Prion Peptide PrP106-126

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Transmissible spongiform encephalopathies are characterised by the transformation of the normal cellular prion protein (PrP^{C}) into an abnormal isoform (PrP^{TSE}). Previous studies have shown that N-methyl-D-aspartate (NMDA) receptor antagonists can inhibit glutathione depletion and neurotoxicity induced by PrP^{TSE} and a toxic prion protein peptide, PrP106-126, in vitro. NMDA receptor activation is known to increase intracellular accumulation of Ca^{2+}, resulting in up-regulation of arachidonic acid (AA) metabolism. This can stimulate the lipoxygenase pathways that may generate a number of potentially neurotoxic metabolites. Because of the putative relationship between AA breakdown and PrP106-126 neurotoxicity, we investigated AA metabolism in primary cerebellar granule neuron cultures treated with PrP106-126. Our studies revealed that PrP106-126 exposure for 30 min significantly up-regulated AA release from cerebellar granule neurons. PrP106-126 neurotoxicity was mediated through the 5-lipoxygenase (5-LOX) pathway, as shown by abrogation of neuronal death with the 5-LOX inhibitors quinacrine, nordihydroguaiaretic acid, and caffeic acid. These inhibitors also prevented PrP106-126-induced caspase 3 activation and annexin V binding, indicating a central role for the 5-LOX pathway in PrP106-126-mediated proapoptosis. Interestingly, inhibitors of the 12-lipoxygenase pathway had no effect on PrP106-126 neurotoxicity or proapoptosis. These studies clearly demonstrate that AA metabolism through the 5-LOX pathway is an important early event in PrP106-126 neurotoxicity and consequently may have a critical role in PrP^{TSE}-mediated cell loss in vivo. If this is so, therapeutic intervention with 5-LOX inhibitors may prove beneficial in the treatment of prion disorders. J. Neurosci. Res. 65:565–572, 2001.

Key words: spongiform encephalopathy; arachidonic acid; annexin V; caspase; apoptosis

Transmissible spongiform encephalopathies (TSE) are a group of neurodegenerative disorders characterised by neuronal cell loss, gliosis, and formation of extracellular deposits of an abnormal conformer of the prion protein (PrP). The function of the normal cellular form of the prion protein (PrP^{C}) has not been clearly elucidated, but PrP knockout ($PrP^{-/-}$) mice do not display any gross defect (Bueler et al., 1992). However, $PrP^{-/-}$-derived cells exhibit deficits in their ability to cope with oxidative stress and copper toxicity (Brown et al., 1997a, 1998; White et al., 1999). The neurotoxicity of an infectious abnormal PrP isoform (PrP^{TSE}) may be the result of a combination of direct neurotoxic effects induced by PrP^{TSE} in conjunction with the loss of normal PrP^{C} function. The presence of PrP^{C} is required for infection, insofar as $PrP^{-/-}$ mice show complete resistance to PrP^{TSE}. $PrP^{-/-}$ neurons are also refractory to toxicity induced by PrP^{TSE} and the neurotoxic prion peptide PrP106-126 (Bueler et al., 1993; Brown et al., 1994; Sailer et al., 1994; Brandner et al., 1996). The PrP106-126 peptide has been extensively used to model the neurotoxic actions of PrP^{TSE} (Forloni et al., 1993). The validity of this is supported by the specificity of the peptide's activity, in that it requires the expression of cellular PrP by the host cell to be neurotoxic (Brown et al., 1994). In contrast, other neurotoxic PrP peptide fragments have not shown this specificity (Haik et al., 2000).

PrP^{TSE} and PrP106-126 neurotoxicity in wild-type neurons is characterised by the activation of N-methyl-D-aspartate (NMDA) receptors. NMDA receptor antagonists such as MK801, memantine, and flupirtine can block PrP^{TSE} and PrP106-126 neurotoxicity (Müller et al., 1993; Perovic et al., 1995, 1997). NMDA receptor activation may result in the up-regulation of the arachidonic acid (AA) pathway (Dumuis et al., 1988). AA metabolites can affect cellular metabolism, including increased oxidative stress through the production of free radicals and up-regulation of excitotoxic stress through the 5-lipoxygenase (5-LOX) pathway and

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oxidative glutamate toxicity (Traystman et al., 1991; Peterson et al., 1995; Li et al., 1997). AA may act directly on glial cells to decrease astrocytic glutamate uptake and potentiating NMDA receptor-mediated Ca^{2+} influx (Barbour et al., 1989; Miller, 1992).

Glial cell activation (gliosis) is an important characteristic of prion diseases, with microglia and astrocytes considered to be necessary for neurotoxicity (Brown et al., 1996). This may be mediated by increases in inflammatory cytokines, such as interleukin (IL)-1β , IL-6, and possibly tumor necrosis factor (TNF)-α and nuclear factor (NF)-κβ, released by microglia (Kim et al., 1999; Peyrin et al., 1999). These factors can up-regulate the AA catabolic pathways (Dayton and Major, 1996; Stella et al., 1997) and cause the observed increase in cyclooxygenase-2 (COX-2) expression, an enzyme involved in AA metabolism (Walsh et al., 2000). Furthermore, the COX-2 pathway mediates the production of the proinflammatory prostaglandins as observed in a murine model of scrapie (Williams et al., 1994, 1997).

Considering the possible role of AA metabolism in prion diseases, we investigated whether the AA pathway and subsequent metabolites are involved in PrP106-126-induced neurotoxicity in vitro. We found that PrP106-126 induced AA release from cerebellar granule neurons (CGN), whereas inhibition of the 5-LOX pathway significantly decreased the neurotoxicity of the peptide in CGN cultures. Inhibitors of the 5-LOX pathway were shown to abrogate significantly expression of apoptotic cell death markers induced by PrP106-126. These findings suggest that perturbed AA metabolism may be an important feature of neurodegeneration in prion diseases. If this is so, therapeutic strategies targeted at the AA or 5-LOX pathways could be beneficial in the treatment of these disorders.

MATERIALS AND METHODS

Reagents

Poly-D-lysine, 3,[4,5 dimethylthiazol-2yl]-2,5 diphenyltetrazolium bromide (MTT), cytosine arabinofuranoside (AraC), quinacrine, propyl gallate, indomethacin, caffeic acid, N-acetyl cysteine, baicalein, nordihydroguaiaretic acid (NDGA), memantine, and buthionine sulfoximine (BSO) were purchased from Sigma (St. Louis, MO). Glutamine, glucose, and gentamycin sulfate were obtained from Gibco BRL (Grand Island, NY). Foetal calf serum (FCS) was obtained from the Commonwealth Serum Laboratories (Melbourne, Australia). The PrP106-126 and scrambled peptide were produced in our laboratory as previously described (Jobling et al., 1999). Caspase activation assay reagents were purchased from Calbiochem-Novabiochem (Darmstadt, Germany).

Primary Neuronal Cultures

Wild-type mice (WT) were C57BL/6J × 129/Sv, which is matched to the genetic background of the PRNP knockout mice previously used to show the specificity of PrP106-126 peptide neurotoxicity (Jobling et al., 1999). Primary neuronal cultures of CGN were established from the WT mice as previously described (White et al., 1998). Briefly, cerebella from postnatal day 4 or 5 (P4–5) mice were removed, dissected free of meninges, and dissociated in 0.025% trypsin. Cells were then plated at a density of 350,000 cells/cm^2 onto poly-D-lysine (5 μg/ml)-coated 48 and 24 well plates (Costar, Cambridge, MA) or 96 well plates (Packard Bioscience, Meriden, CT). CGN were cultured in BME (Gibco BRL) supplemented with 10% FCS, 2 mM glutamine, and 25 mM KCl.

Treatment of Cultures With PrP106-126

Unless otherwise stated, PrP106-126 was diluted in culture medium (BME) and added to the neuronal cultures at a final concentration of 80 μM on day 1 in vitro and repeated at day 3 in vitro. Cell viability was determined on day 5 in vitro. These conditions are similar to those previously used, with the exception that peptide treatment was continued only to day 5 in vitro, rather than the longer period of exposure previously employed (Forloni et al., 1993; Brown et al., 1994; Jobling et al., 1999). This provided a lower level of cell death for investigation of the early events involved in the PrP106-126 neurotoxic pathway.

Treatment of Cells With Inhibitors of Arachidonic Acid Metabolism

The highest possible concentrations of inhibitors that did not affect cell viability were determined prior to experiments with PrP106-126 (data not shown). These were determined using previously published concentration ranges for these AA pathway inhibitors (Barbour et al., 1989; Oomagari et al., 1991; Li et al., 1997; Luo et al., 1998). Cultures were exposed to inhibitors on day 1 in vitro, 15 min before the addition of PrP106-126. Concentrations of inhibitors used were 1 μM quinacrine, 2 μM indomethacin, 10 μM propyl gallate, 4 μM caffeic acid, 2 μM baicalein, 150 μM N-acetyl cysteine, and 4 μM NDGA.

Measurement of Cell Viability

The MTT assay was used to measure cell viability unless otherwise stated. Culture media was replaced with 0.32 mg/ml MTT in Locke's buffer (154 mM NaCl, 5.6 mM KCl, 2.3 mM $CaCl_2$, 1 mM $MgCl_2$, 3.6 mM $NaHCO_3$, 5 mM HEPES, and 5.6 mM glucose, pH 7.4) at 37°C for 1–2 hr. The MTT was removed, and cells were solubilized with dimethyl sulfoxide. Samples were measured in triplicate using a spectrophotometer at 570 nm. All MTT viability measurements are taken as a percentage of the untreated control.

Measurement of [^{3}H]AA Release

Cerebellar granule neurons were plated onto Viewplate 96 well plates (Packard Bioscience). The medium was replaced with fresh BME containing 0.5 μCi [^{3}H]AA on day 3 in vitro and incubated for 24 hr at 37°C. Labelling medium was removed, and the cells were thoroughly washed with unlabelled culture media and exposed to either 80 μM scrambled PrP106-126 or 80 μM WT PrP106-126 and/or 1 μM quinacrine or 50 μM memantine in unlabelled media for 30 min. Medium was then removed to a new plate, cells were lysed with 0.5 M NaOH, and scintillant was added to both lysed cells and media samples. The level of cellular and released [^{3}H]AA was measured using a Top Count scintillation counter (Packard Instrument).

[^{3}H]AA release was calculated as the percentage of [^{3}H]AA released into the supernatant compared with the total [^{3}H]AA taken up by the cells.

Effect of Inhibitors on PrP106-126-Induced Caspase Activation

Apoptosis was measured using a colorimetric assay for caspase 3-like activity. Cultures were washed twice with Locke's buffer before being extracted at 4°C into extraction buffer (20 mM Tris/HCl, 1 mM EDTA, 0.25 M sucrose, 1% Triton X-100, 1 mM DTT, 0.5 mM PMSF, 1 μg/ml pepstatin, 1 μg/ml aprotintin). Extract supernatant was collected and assayed for caspase activity through hydrolysis of the caspase 3 substrate DEVD-pNA (Calbiochem-Novabiochem), resulting in the formation of chromogenic pNA at 37°C for up to 24 hr. The formation of pNA was measured in triplicate using a spectrophotometer at 405 nm.

Effect of Inhibitors on PrP106-126-Induced Annexin V Binding

Cerebellar granule neurons were exposed to PrP106-126 and AA pathway inhibitors as described above. Medium was removed after 24 hr, and the cells were thoroughly washed before being incubated with annexin V-fluorescein isothiocyanate (FITC) conjugate (a generous gift from Dr. Shafiq Ahmed, Walter and Eliza Hall Institute of Medical Research, Parkville, Australia) and 10 μg/ml propidium iodide for 30 min before being washed again. Cells were photographed using a Leica DMIRB/E inverted fluorescent microscope.

Statistical Analysis

All experiments were replicated on at least four occasions, the data from which were analysed using ANOVA and Newman Keuls tests to determine statistical significance.

RESULTS

PrP106-126 Induces AA Release From CGN: Inhibition by Cotreatment With Quinacrine or Memantine

To investigate the role of AA metabolism in PrP106-126 neurotoxicity, we measured the level of [^{3}H]AA release induced by 80 μM PrP106-126 in primary CGN. This concentration of PrP106-126 has previously been shown to induce approximately 40% neuronal death in our CGN cultures after 5 days of exposure (Jobling et al., 1999). The addition of 80 μM PrP106-126 to [^{3}H]AA-labelled CGN cultures for 30 min resulted in a greater than 100% increase in [^{3}H]AA release from 1.2% to 2.6% of total cellular counts ($\star P < 0.05$; Fig. 1A). This level of AA release is comparable to that reported for other neurotoxic mediators of AA release (Dumuis et al., 1988; Oomagari et al., 1991; Himmelseher et al., 1996). This clearly demonstrated that neuronal AA metabolism is rapidly up-regulated following PrP106-126 treatment. The specificity of the response was established by the PrP106-126 scrambled peptide not increasing [^{3}H]AA release above basal levels (Fig. 1A).

To confirm that PrP106-126 interacted with the AA pathway, we examined the effect of quinacrine, an inhibitor of phospholipase A2 (PLA2), on PrP106-126-mediated AA release. PLA2 is the key enzyme involved in the release of AA from the membrane (see Fig. 6). [^{3}H]AA-labelled CGN were exposed to 1 μM quinacrine 15 min prior to PrP106-126 treatment (80 μM), and [^{3}H]AA release was determined after a further 30 min. This treatment completely inhibited PrP106-126-mediated AA release compared with PrP106-126 alone ($\star P < 0.05$; Fig. 1B). This confirmed that PrP106-126 can induce a rapid up-regulation of AA release in CGN cultures. To characterise further the pathway of PrP106-126-mediated AA release, we treated CGN cultures with memantine, an NMDA receptor antagonist previously shown

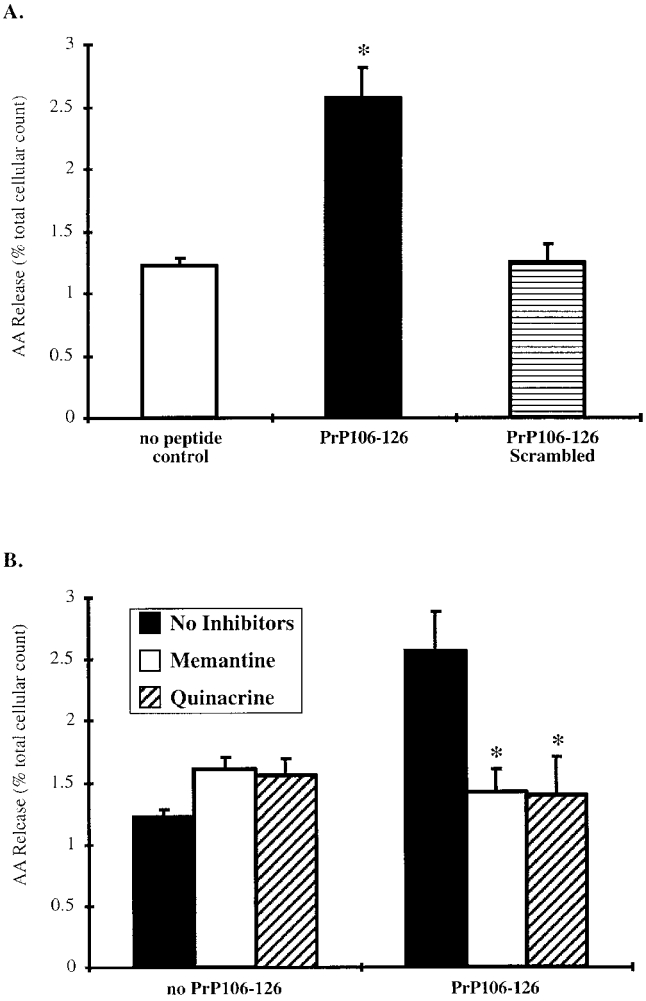

Fig. 1. Quantification of [^{3}H]AA release from cerebellar granule neurons after treatment with PrP106-126 or the scrambled peptide (**A**). The addition of PrP106-126 caused a significant increase in [^{3}H]AA release (as a percentage of total cellular counts), which was not exhibited in the cells treated with the scrambled peptide ($\star P < 0.05$). This increase in [^{3}H]AA release mediated by the addition of PrP106-126 was abolished when the cells were preincubated with either 1 μM quinacrine, a PLA2 inhibitor, or 50 μM memantine, an NMDA receptor antagonist (**B**; $\star P < 0.05$).

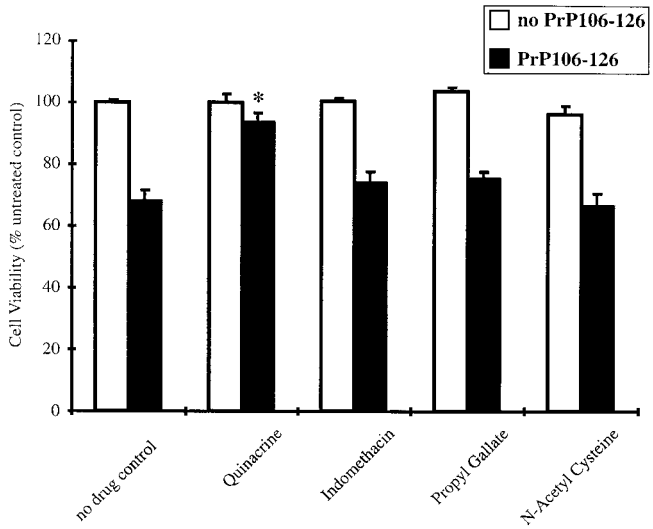

Fig. 2. Measurement of cell viability using the MTT reduction assay on CGN cultures after treatment with PrP106-126 and inhibitors of the AA pathways or with the antioxidants propyl gallate and N-acetyl cysteine. The decrease in cell viability mediated by PrP106-126 was significantly abrogated by 1 μM quinacrine ($\star P < 0.05$). However, the addition of the COX inhibitor indomethacin or the addition of the antioxidants had no effect on the PrP106-126-mediated neurotoxicity. Inhibitors alone had no effect on cell viability.

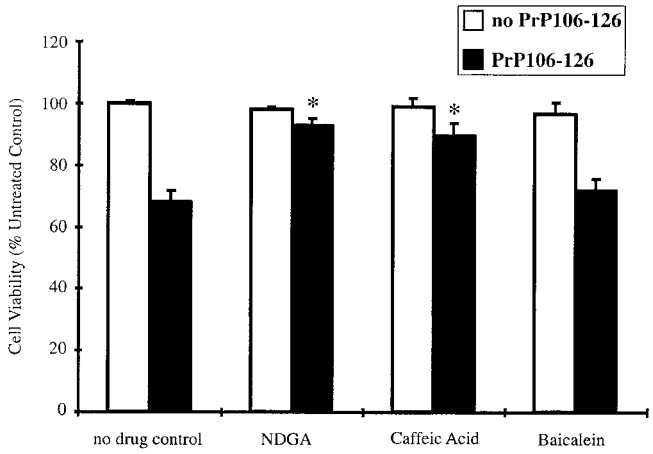

Fig. 3. Measurement of cell viability using the MTT reduction assay on CGN cultures after treatment with PrP106-126 and inhibitors of the LOX pathways, 4 μM NDGA, 4 μM caffeic acid, or 2 μM baicalein. The PrP106-126-mediated neurotoxicity was ameliorated by the addition of either NDGA or caffeic acid ($\star P < 0.05$). Inhibitors alone had no effect on cell viability.

to block PrP^{TSE} neurotoxicity (Müller et al., 1993). The [^{3}H]AA-labelled CGN were treated with 50 μM memantine 15 min prior to adding 80 μM PrP106-126, and AA release was determined after a further 30 min. Memantine decreased [^{3}H]AA release to basal levels ($\star P < 0.05$; Fig. 1B), indicating that PrP106-126-mediated [^{3}H]AA release may occur through NMDA receptor activation.

Inhibitors of the 5-LOX Pathway Decrease PrP106-126 Neurotoxicity

The release of AA by PrP106-126 suggests that AA metabolites may be involved in PrP106-126-mediated neurotoxicity. To investigate this, we examined the effect of AA catabolic pathway inhibitors (see Fig. 6) on PrP106-126 toxicity. The addition of 1 μM quinacrine 15 min prior to adding 80 μM PrP106-126 significantly inhibited PrP106-126-mediated cell death ($\star P < 0.05$; Fig. 2). In contrast, indomethacin (2 μM), an inhibitor of the cyclooxygenase pathways, had no effect on cell viability. This indicates that AA metabolites of the cyclooxygenase pathway do not modulate PrP106-126 toxicity. Because AA metabolism can increase oxidative stress levels, we tested the effect of antioxidants on PrP106-126 toxicity. The addition of the antioxidants 10 μM propyl gallate or 150 μM N-acetyl cysteine together with PrP106-126 had no effect on PrP106-126-mediated cell death (Fig. 2).

These data indicate that inhibiting AA production blocks PrP106-126-mediated neurotoxicity through an alternative pathway to the cyclooxygenase. Therefore, we examined the role of the LOX pathways of AA catabolism for their ability to modulate PrP106-126 toxicity. The addition of the general LOX inhibitor NDGA (4 μM) 15 min prior to adding 80 μM PrP106-126 increased cell viability by 24% ± 2.6% compared with PrP106-126 alone ($\star P < 0.05$; Fig. 3). To define which arm of the LOX pathway was involved, we tested caffeic acid, a 5-LOX specific inhibitor. The addition of 4 μM caffeic acid significantly increased cell viability by 21% ± 4.3% ($\star P < 0.05$; Fig. 3). In contrast, the 12-LOX-specific inhibitor baicalein (2 μM) had no effect on cell viability. These findings demonstrate a clear role for the 5-LOX but not the 12-LOX pathway of AA metabolism in PrP106-126-mediated neurotoxicity.

PrP106-126-Induced Annexin V Binding Is Reduced by 5-LOX Inhibitors

Annexin V binding is an established early marker of apoptosis (for review see Allen et al., 1997). We have previously found that PrP106-126 treatment rapidly upregulates annexin V binding in primary neuronal cultures, indicating a central role for apoptotic activation in PrP106-126 neurotoxicity (White et al., 2001). To examine whether the neurotoxic effects induced by PrP106-126 via the 5-LOX pathway involve proapoptotic changes, we measured annexin V binding in PrP106-126-treated cultures using an annexin V-FITC conjugate. Consistently with our previous findings, we observed a substantial increase in annexin V-FITC staining in CGN cultures exposed to 80 μM PrP106-126 for 24 hr (Fig. 4). Treating the CGN cultures with NDGA (4 μM) or caffeic acid (4 μM) resulted in a marked decrease in annexin V binding compared with PrP106-126 alone (Fig. 4). In contrast, indomethacin and baicalein had little affect on annexin V staining induced by PrP106-126 in CGN cultures. The cultures were also stained with propidium

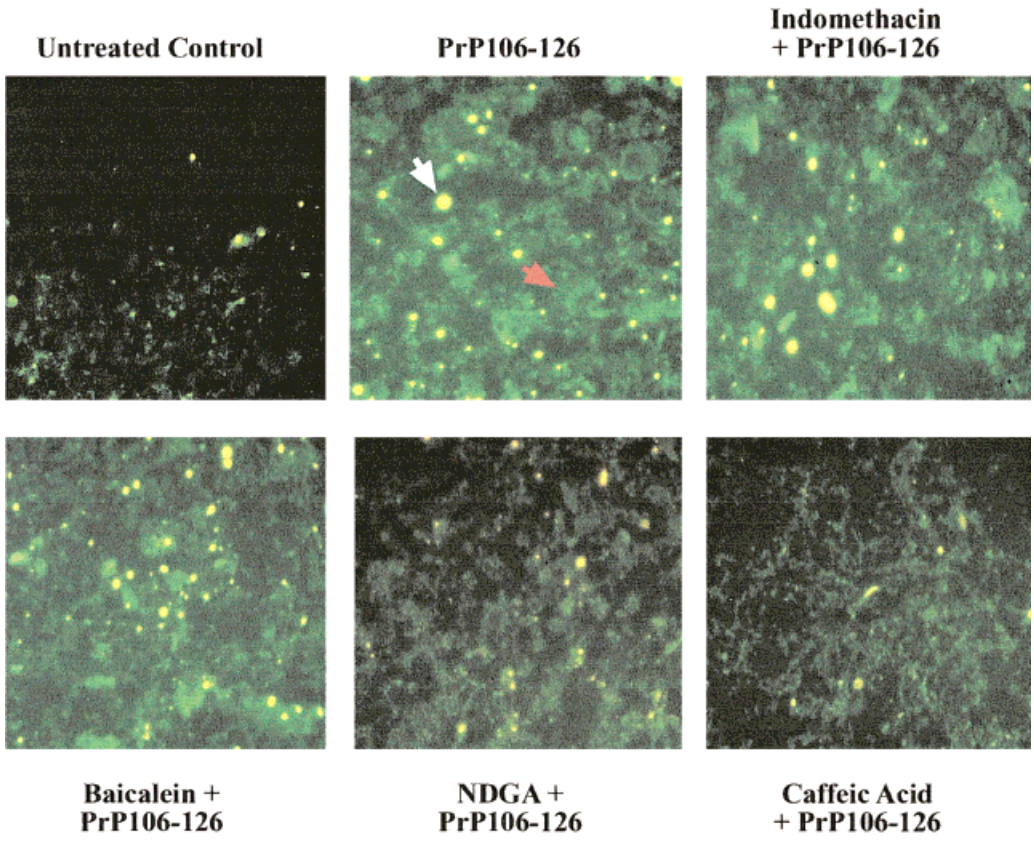

Fig. 4. Visualisation of annexin V binding to CGN cultures in early-stage apoptosis using an FITC label in conjunction with propidium iodide to detect lysed cells. The yellow propidium iodide staining is indicated by a white arrow, and the green annexin V staining is indicated by a red arrow. The untreated control shows a small degree of necrotic cell death, as expected in a primary neuronal culture. In comparison, the PrP106-126-treated cultures showed a significantly higher degree of staining. Similarly, the cultures pretreated with indomethacin and baicalein before treatment with PrP106-126 showed a high level of annexin V binding. However, the cultures pretreated with NDGA and caffeic acid before treatment with PrP106-126 showed a substantially reduced level of staining. All cultures were pretreated for 15 min with inhibitors before the addition of PrP106-126 for 24 hr prior to annexin V-FITC staining of unfixed cultures.

iodide, which labels the nucleus of dead cells. Consistently with the annexin V data, there was a decrease in the number of propidium iodide-staining cells following treatment with NDGA and caffeic acid. These findings indicate that up-regulation of the 5-LOX pathway by PrP106-126 promotes early apoptotic marker expression in CGN.

PrP106-126-Mediated Caspase 3-Like Activation Is Abrogated by 5-LOX Inhibitors

To quantitate the relationship between AA metabolism and PrP-mediated activation of proapoptotic markers, we measured the activity of neuronal caspase 3 in these cultures. We chose caspase 3 because it is a central effector protease activated in the early stages of apoptosis (Schulz et al., 1999). We have previously found that caspase 3 activity is elevated in cortical neurons treated with PrP106-126 and correlates with other methods of measuring caspase 3 activation, such as the use of an antiactive caspase 3 western blot (White et al., 2001). Furthermore, the level of caspase 3-like activation mediated by PrP106-126 is at its optimal level after 24 hr (White et al., 2001). CGN cultures were exposed to PrP106-126 (80 μM) for 24 hr, and caspase 3-like activity was determined in cell lysates by cleavage of the substrate DEVD-pNA. PrP106-126 alone induced a significant increase in CGN caspase 3-like activity of 20% ± 3.7% compared to untreated neurons ($\star P < 0.05$; Fig. 5). Treating the cultures with quinacrine

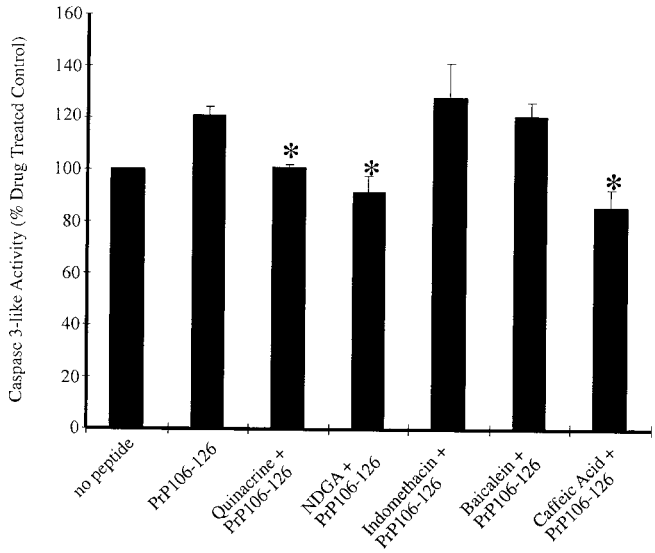

Fig. 5. Measurement of caspase 3-like activity exhibited by PrP106-126-treated CGN cultures after 24 hr. Data were taken as percentage of drug-treated control. A substantial increase in caspase 3-like activity was observed after treatment with PrP106-126. The PrP106-126-induced caspase 3-like activation was significantly decreased by the pretreatment of CGN cultures with 1 μM quinacrine, 4 μM NDGA, or 4 μM caffeic acid ($\star P < 0.05$).

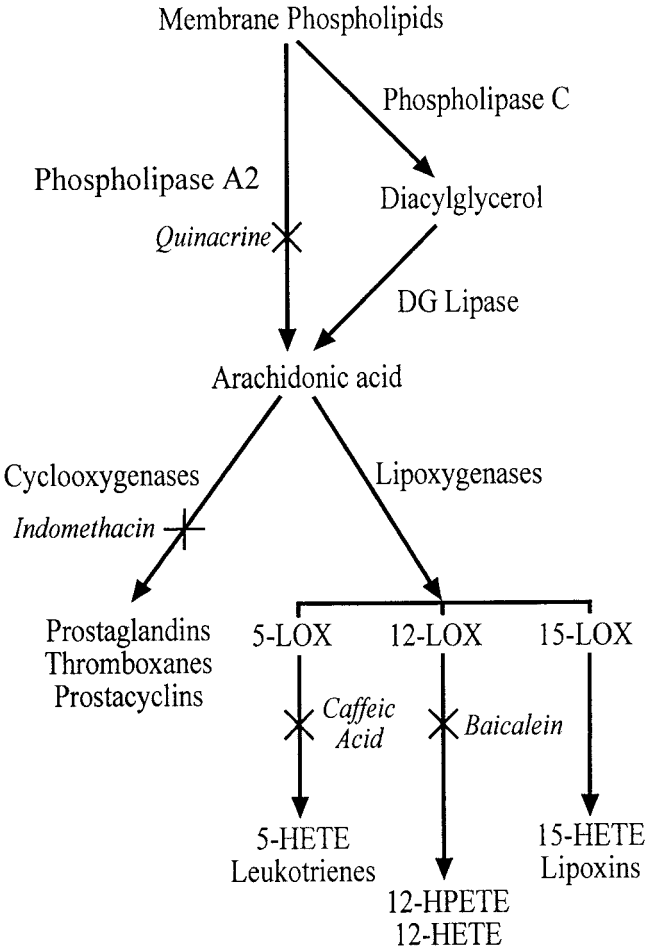

Fig. 6. Diagram showing the production of AA from membrane phospholipids through either phospholipase A2- or phospholipase C-mediated pathways. AA is then metabolised via either the cyclooxygenase or the lipoxygenase pathway. The primary site of action for each of the inhibitors is indicated in italic.

(1 μM), NDGA (4 μM), or caffeic acid (4 μM) reduced caspase 3-like activation to background control levels ($\star P < 0.05$; Fig. 5). In contrast, baicalein (2 μM) and indomethacin (2 μM) had no effect on PrP106-16-induced caspase activation (Fig. 5). This is consistent with the annexin V binding and propidium iodide results and demonstrates a specific role for the 5-LOX pathway in PrP106-126-associated activation of apoptosis.

DISCUSSION

The involvement of the AA pathway (summarised in Fig. 6) and its metabolites in PrP neurotoxicity has previously been reported with a murine scrapie model (Walsh et al., 2000). There was an up-regulation of COX-2 and increased production of prostaglandin-2 by astrocytes from scrapie-infected murine brain (Williams et al., 1994, 1997; Walsh et al., 2000). This is consistent with other studies that have shown increased production of proinflammatory cytokines such as IL-1β and IL-6 by microglia in prion diseases (Campbell et al., 1994; Kim et al., 1999; Peyrin et al., 1999). However, despite a report that NMDA receptor antagonists can block the neurotoxicity of PrP^{TSE} and PrP106-126 in vitro, no further studies have been reported on the involvement of the AA pathway in this toxic process (Müller et al., 1993). The present study was designed to determine whether AA metabolism is induced by PrP106-126 in CGN, and, if so, whether this has a central role in the neurotoxic action of the peptide. The treatment of CGN cultures with inhibitors of AA metabolism enabled us to identify a key role for the 5-LOX pathway in PrP106-126 neurotoxicity. The involvement of the AA metabolic pathways in PrP toxicity has important implications for understanding the neurotoxic processes involved in prion diseases.

We demonstrated that PrP106-126 induces [^{3}H]AA release from CGN cultures in vitro in a manner that could not be replicated using the scrambled version of the peptide, indicating the specificity of the response. The PrP106-126-mediated AA release could be inhibited by pretreating the cells with the PLA2 inhibitor quinacrine or the NMDA receptor antagonist memantine. This indicates that PrP106-126 may promote activation of PLA2, through increased intracellular Ca^{2+} levels mediated by activation of NMDA receptors (Dumuis et al., 1988). This is supported by previous studies demonstrating elevated intracellular Ca^{2+} levels in neurons treated with PrP106-126 (Brown et al., 1997b; Kawahara et al., 2000). We then revealed that quinacrine, NDGA (a general LOX inhibitor), and caffeic acid (a 5-LOX-specific inhibitor) were able to suppress PrP106-126 neurotoxicity in vitro. However, indomethacin, a cyclooxygenase inhibitor, and baicalein, a 12-LOX inhibitor, did not affect PrP106-126 toxicity, demonstrating a specific role for the 5-LOX pathway. Intriguingly, the antioxidants propyl gallate and N-acetyl cysteine had no effect on the toxicity of the peptide in our study, suggesting that the protective effect mediated by the 5-LOX inhibitors is not due directly to elevation of antioxidant levels.

Although the 12-LOX enzyme is considered to be the most abundant in the brain, both 5-LOX and its activating enzyme, the 5-lipoxygenase activating protein (FLAP), are expressed at their highest levels in the cerebellum and hippocampus (Lammers et al., 1996). Therefore, it is appropriate to use cerebellar granule neuronal cultures to study the potential interactions between 5-LOX and PrP106-126 neurotoxicity (Manev and Uz, 1999). The 5-LOX enzyme has also been observed in the pineal gland, where it is believed to act in conjunction with 12-LOX to mediate circadian functions of the pineal gland (Vacas et al., 1987; Kawajiri et al., 1997; Uz and Manev, 1998). 5-LOX acts by forming 5-hydroperoxyeicosatetraenoic acid (5-HPETE) as its primary product. This is converted to either 5-hydroeicosatetraenoic acid (5-HETE) or leukotriene A_4 (LTA_4) and may then be converted into either LTB_4 by an LTA_4 hydrolase or into LTC_4 by a

glutathione-S-transferase, using glutathione as a substrate (Morris and Rodger, 1998). The many effects of the 5-LOX pathway products within the brain have yet to be clearly elucidated. However, the 5-LOX pathway has been implicated in kainate-associated excitotoxic injury (Simmet and Tippler, 1990).

Furthermore, 5-LOX may increase the K^+-evoked release of the excitotoxic amino acid aspartate from hippocampal slices (Peterson et al., 1995). This represents a possible neurotoxic mechanism, insofar as excitotoxicity has been implicated in PrP106-126 neurotoxicity (Brown, 1999). It has also been found that astrocytes from PrP knockout mice show a decreased level of glutamate uptake (Brown and Mohn, 1999). Interestingly, caffeic acid has been shown previously to be effective in reducing glutamate receptor-mediated neurotoxicity in vivo (Uz et al., 1998). 5-LOX is believed to be involved in the increased release of γ-aminobutyric acid (GABA) and acts synergistically with other second messengers, such as protein kinase C (PKC), in the release of luteinising hormone (Kiesel et al., 1991; Peterson et al., 1995). This activity may be of particular significance given the up-regulation of PKC in astrocytes as a response to PrP106-126, suggesting a relationship between PrP106-126, 5-LOX, and PKC activity (Combs et al., 1999). Other actions of 5-LOX pathway products include the prolonged excitation on cerebellar Purkinje cells and the mediation of the effects of somatastatin on pyramidal neurons in the hippocampus (Schweitzer et al., 1993; Lammers et al., 1996).

Interestingly, there is an increased level of 5-LOX expression in the brains of elderly subjects, which may be mediated through decreasing levels of melatonin (Uz et al., 1997; Manev et al., 2000). Aged rats with increased levels of 5-LOX mRNA transcription were more susceptible to kainate-induced excitotoxic injury, which was ameliorated by the 5-LOX inhibitor caffeic acid (Uz et al., 1998). These observations suggest that increases in 5-LOX expression in the aging brain may increase the level of neuronal vulnerability to excitotoxic insult and possibly neurodegenerative diseases, such as prion diseases and Alzheimer's disease. The high level of 5-LOX activity in the cerebellum and our findings that 5-LOX is critical to PrP106-126-induced apoptosis and loss of cell viability in CGN cultures indicates that further investigation of this complex pathway in prion diseases is warranted. These studies also identify the 5-LOX pathway as a potential target for in vivo treatment of prion disorders through inhibition of AA-associated pathways.

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