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THE PRION PROTEIN AND CELLULAR CHOLESTEROL HOMEOSTASIS

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The amount of lipids in cell membranes seems to regulate the interaction of the prion protein with cells and the propagation of prions. We investigated how the synthetic human prion peptide PrP 106-126 affected the chemico-physical and biochemical properties of nerve and HL60 cells. PrP 106-126 rapidly increased cell membrane microviscosity, inhibited cellular cholesterol release and increased membrane cholesterol content. PrP also inhibited cellular 3-hydroxy-3-methylglutaryl-coenzyme A reductase activity. These findings indicate that PrP 106-126 alters cellular cholesterol homeostasis and may help clarify how changes in membrane lipid composition are involved in the progression of prion encephalopathies.

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INTRODUCTION

The mechanism of neurodegeneration caused by the abnormal isoform of the normal cellular prion protein (PrPC) is not fully understood. This abnormal isoform (also known as PrPSc^{1,2}) accumulates in the brain of humans and animals with prion diseases. Cellular plasma membranes play a large role in prion disease and prions propagation. In scrapie-infected brains, PrPSc is associated with the cell membrane fraction.^{3,5} In

cultured nerve cells PrPSc causes membrane protein alterations, increased membrane microviscosity and abnormal receptor-mediated calcium ion responses.³⁻⁷ Cell membranes, particularly the caveolae cholesterol- and sphingolipid-rich portions, seem important in regulating the conversion of PrPC to PrPSc,⁸ both being found in caveolae-like domains purified from scrapie infected cells.⁹⁻¹⁰ The protein-membrane interaction, especially with cholesterol-containing membranes, may regulate the formation of PrPSc and their participation in prions propagation. In fact, a reduction of cellular cholesterol levels, critical for caveolae architecture, inhibited the conversion of PrPC to PrPSc.^{6,9}

To better investigate the relationship between prion proteins and membrane lipid composition, we used the synthetic prion peptide PrP 106-126. This sequence comprises residues 106-126 of human PrP and corresponds to a highly conserved region of the protein located in the flexibly disordered N-terminal domain. This protein region is believed to undergo a profound conformational change in the conversion from PrPC to PrPSc¹¹ and in PrPSc amyloid formation.^{12,13} PrP 106-126 displays some of the pathogenic and physico-chemical properties of PrPSc.¹⁴⁻¹⁶ Like PrPSc, it causes apoptosis of hippocampal neurons and induces hypertrophy and proliferation of astrocytes in vitro.¹⁶ It also readily forms amyloid-like fibrils that are partially resistant to the action of proteases.¹⁶ These properties make it useful in vitro for investigating of the mechanisms of prion-related encephalopathies.

We previously studied membrane homeoviscosity of the HL60 cells and found that HL60 cells are very sensitive to changes in the membrane

cholesterol content and thus represent a good model for investigating the effects of cholesterol on cell responses.¹⁷ Here we designed experiments to clarify whether PrP 106-126 affects the homeoviscosity and cholesterol content of primary neurons (sensitive to the toxic action of the peptide¹⁵⁻¹⁶) compared to HL60 cells, which are resistant to the peptide toxic effect.

MATERIALS AND METHODS

Materials. The prion protein fragments PrP 106-126 and PrP 106-126-scrambled were synthesized as described elsewhere.¹⁶ Crude peptides were washed several times with diethylether and purified by reverse-phase HPLC and capillary electrophoresis (Quanta 4000-Millipore, MA, USA). Purity was greater than 95 % for both peptides.¹⁶ After lyophilization, powder peptides were stored at -80°C until use. Before each experiment, PrP 106-126 and PrP 106-126 scrambled powders were weighed and dissolved in deionized water at 1 mg/ml (stock solution), and added to the medium to obtain the required concentration. Any excess stock solution was eliminated.

The fluorescent probe 1,6-diphenyl-1,3,5-hexatriene (DPH) was purchased from Janssen (Beerse, Belgium) and 1-(4-trimethylammonium phenyl)-6-phenyl-1,3,5-hexatriene-p-toluene sulfonate salt (TMA-DPH) was from Kodak (Rochester, USA). Fetal bovine serum (FBS) and culture media were from Labtek (France). [^{14}C]Cholesterol (specific activity 50-62 Ci/mmol) and [^{14}C]hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA; specific activity 50-62 Ci/mmol) were from Amersham (Little Chalfont, UK). Rhodamine G and [^3H]5-mevalonolactone (specific activity 10-40 Ci/mmol) were from Sigma Chemical Co (St. Louis, USA).

Cells. Nerve cells were obtained from rat brains on embryonic day 17 and cultured in DMEM medium containing 10 % FBS.¹⁶ Human promyelocytic leukemia HL60 cells were obtained from the American Type Culture Collection (Rockville, MD) and cultured in RPMI 1640 medium containing 10 % FBS.

To obtain HL60 cells with a high cholesterol concentration (HL60-Chol), cells (2×10^4 /ml) were incubated for 48 h in 10 % FBS RPMI medium containing cholesterol at a concentration of 25 $\mu\text{g}/\text{ml}$ (ethanol stock solution).¹⁷ This dose was chosen because it significantly increased the cell cholesterol content without affecting phospholipid concentration and cell viability.¹⁷ The ethanol

concentration did not exceed 0.5 % in the final experimental setting.

Acute cytotoxicity experiments were done on nerve cells (1×10^6 cells/well) and on HL60 cells (1×10^6 /ml) on day 5 of culture, by exposing them to 25-50 μM PrP 106-126 and scrambled PrP 106-126 (freshly dissolved at 1 mg/ml in deionized water). Cell viability was determined 24 h later. Chronic cytotoxicity was determined on nerve (1×10^6 cells/well) and HL60 (1×10^6 /ml) cells by the addition of 5-50 μM PrP 106-126 and scrambled PrP 106-126 on days 1, 3 and 5 of culture and evaluating cell viability on day 7. Viability was quantitatively assessed by the MTT method with an automated micro-plate reader (Perkin Elmer).

Membrane microviscosity. The effect of peptides on overall membrane microviscosity was assessed using DPH as a fluorescent probe.¹⁸ PrP 106-126 and scrambled PrP 106-126 were freshly dissolved at 1 mg/ml in deionized water. Rat nerve (1×10^6 /well) and HL60 cells (1×10^6 /ml) were then incubated at 37°C for 24 h with 1-50 μM of each peptide. At the end of incubation, nerve cells were detached by trypsinization and gently centrifuged at 550 x g for 10 min; HL60 cells were directly centrifuged at 550 x g for 10 min and washed with saline. Cells were then resuspended in 2 μM of DPH in Dulbecco's phosphate (5 mM) buffered saline (PBS), pH 7.4, and incubated for 30 min at room temperature. The fluorescence polarization (FP) value of treated and untreated cells was determined at $25 \pm 1^{\circ}\text{C}$ using a MV-1 microviscosimeter (Elscont, Haifa, Israel).

The effect of peptides on plasma membrane microviscosity was determined using TMA-DPH.¹⁹ Briefly, after 24 h incubation of nerve (1×10^6 /well) or HL60 cells (1×10^6 /ml) at 37°C with 50 μM peptide, the cells were centrifuged and washed as described above, resuspended with 1 ml of 0.9 % NaCl and immediately mixed with 1 ml of 1 μM TMA-DPH in 5 mM PBS solution, pH 7.4. The FP value of treated and untreated cells was immediately recorded at $25 \pm 1^{\circ}\text{C}$.

The FP value is a function of the emission light (em, 420 nm), detected through analyzers oriented parallel (FP1) and perpendicular (FP2) to the direction of the polarization of the exciting light (ex, 365 nm), according to the equation $\text{FP} = (\text{FP2} - \text{FP1})/(\text{FP1} + \text{FP2})$.¹⁸ Membrane microviscosity (η , poise) was related to FP according to the equation: $\eta = 2\text{FP}/(0.46 - \text{FP})$.¹⁸

Cholesterol and protein content. Cellular cholesterol and protein content were determined before and after 24 h incubation with 50 μM PrP 106-126 or PrP 106-126-scrambled at 37°C . At the end of

incubation, nerve cells were detached by trypsinization and centrifuged at 550 x g for 10 min; HL60 cells were centrifuged, and then washed with 0.9% NaCl. Total lipids were extracted according to Folch *et al.*²⁰ and cholesterol was determined using an enzymatic assay kit (Boehringer Mannheim, Germany). Cellular protein concentration was determined on cell lysate using the Bio-Rad protein assay (Bio-Rad, Germany).

Cholesterol uptake and release. To assess the effect of PrP 106-126 on cholesterol uptake, HL60 cells (1×10^6 cells/ml) were incubated with 50 μ M PrP 106-126 or PrP 106-126-scrambled for 24 h at 37°C, in the presence of 33.5 μ M of [¹⁴C]cholesterol. Dose-response experiments have shown that this cholesterol concentration was saturating. The cells were then centrifuged at 550 x g for 10 min, washed twice with 5 mM PBS, pH 7.4, and the radioactivity was determined in the cellular pellet using a β -counter scintillator (Beckman, USA). For cholesterol release experiments, HL60 cells (1×10^6 cells/ml) were incubated with 33.5 μ M [¹⁴C]cholesterol for 20 h at 37°C. The cells were then centrifuged at 550 x g for 10 min, washed twice with 5 mM PBS, pH 7.4, resuspended in RPMI 10 % FBS and treated with 50 μ M PrP 106-126 or its scrambled mate. After 24 h at 37°C cells were pelleted at 550 x g for 10 min and the released to the media radioactivity was counted in the supernatant.

HMG-CoA reductase activity. HMG-CoA reductase activity was determined according to Kita *et al.*²¹ Briefly, nerve and HL60 cells (1×10^6 cells/ml) were incubated with 50 μ M PrP 106-126 or PrP 106-126-scrambled for 24 h at 37°C. The cell lysate obtained from these cells (about 50 μ g protein) was then incubated for 30 min at 37°C in a 20 mM imidazole solution, pH 7.4, containing 5 mM dithiothreitol and 5 U alkaline phosphatase (Sigma-Aldrich, EC 3.1.3.1, from Escherichia Coli, catalog number P 4069, 20-50 units/mg protein). [¹⁴C]HMG-CoA (87 μ M, final concentration) was added, and samples were incubated for 1 h at 37°C. The reaction was stopped by adding HCl 18.5 % (wt/vol), with 29 μ M [³H]mevalonolactone as internal standard, and samples were left overnight at 4°C. The samples were then centrifuged at 1000 x g for 20 min, and [³H]mevalonolactone and [¹⁴C]mevalonolactone were copurified by eluting the supernatant on a Kieselgel plate (20 x 20 mm, Merck, Germany) using acetone:toluene (1:1 vol/vol) as mobile phase, and identified using Rhodamine G. The R_f value of the band was assessed by running unlabeled mevalonolactone in parallel. The spots were scraped off and the radioactivity counted. HMG-CoA reductase activity

was expressed as pmol [¹⁴C]mevalonolactone formed/min/mg protein.

Statistical analysis. The data are presented as mean \pm standard deviation. For each experimental value the number of independent cultures/experiments (n) is indicated. Non-parametric Mann-Whitney signed rank test was used for determining significant differences between experimental values. A probability of 0.05 (two-tailed) or less was accepted as statistically significant.

RESULTS AND DISCUSSION

We initially set up experiments to identify the dose of PrP 106-126 that, after 24 h exposure, significantly affect the FP of rat nerve and HL60 cells. Cells were exposed from 1 up to 50 μ M PrP 106-126 or to its scrambled mate for 24 h and the FP value was measured. This dose range is the same used for investigating the toxicity of PrP 106-126 and its ability to rapidly increase the cellular plasma membrane microviscosity.^{15,16} As shown in Figure 1, after 24 h exposure, the rigidifying effect of PrP 106-126 was dose-related. No effect was observed at 1 and 10 μ M of the peptide, and the rigidification become significant starting from 25 μ M, in both nerve and HL60 cells (Figure 1). PrP 106-126 had maximal effect on cell microviscosity at 50 μ M when the FP values rose of 56% in HL60 and 15% in nerve cells (Figure 1, Table 1). PrP 106-126 scrambled had no effect on the FP value of either cell type at any dose (Table 1).

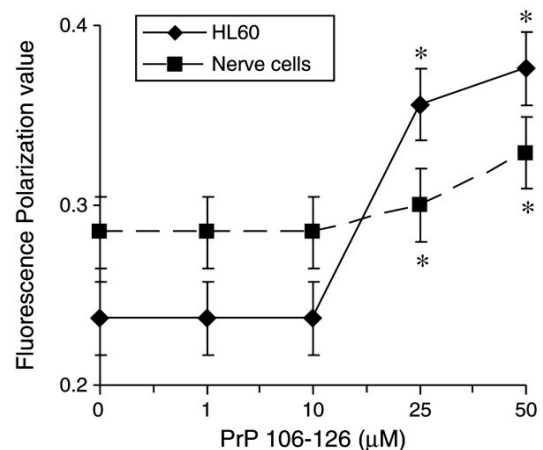


Figure 1. Dose-response effect of 24 h incubation with PrP 106-126 on HL60 and rat nerve cells. Cells (1×10^6 /ml) were incubated at 37°C for 24 h with 1-50 μ M PrP 106-126, then processed as described in the text, gently centrifuged at 550 x g for 10 min, and washed with saline. The effect of the peptide on the overall membrane microviscosity was assessed using 1,6-diphenyl-1,3,5-hexatriene (DPH). The fluorescence polarization (FP) values were determined at 25°C. Asterisk indicates statistically significant difference ($p < 0.01$) compared with untreated cells.

Table 1. Effect of PrP 106-126 on fluorescence polarization and on cellular cholesterol content.**A. HL60 cells**

Treatment	DPH FP Arbitrary unit (%)	TMA-DPH FP Arbitrary unit (%)	Cholesterol ($\mu\text{g}/\text{mg}$ protein)
None	0.237 \pm 0.002 (100)	0.356 \pm 0.003 (100)	43.10 \pm 8
PrP106-126	0.376 \pm 0.008 ** (156)	0.385 \pm 0.004 * (108)	145.10 \pm 9 **
PrP106-126 (scrambled)	0.240 \pm 0.005 (100)	0.360 \pm 0.003 (100)	34.22 \pm 11

HL60 (A) and rat nerve (B) cells (1×10^6 /ml) were incubated at 37°C for 24 h with 50 μM PrP 106-126 or PrP 106-126-scrambled. At the end of incubation, cells were processed as described in the text, gently centrifuged at 550 x g for 10 min, and washed with saline. The effect of the peptide on overall membrane microviscosity was assessed using 1,6-diphenyl-1,3,5-hexatriene (DPH). Cells were resuspended in 2×10^6 M DPH in PBS, pH 7.4, and incubated for 30 min at room temperature. The fluorescence polarization (FP, in arbitrary units) values were determined at 25°C. The effect of peptides on plasma membrane microviscosity was assessed by using 1-(4-trimethylammonium phenyl)-6-phenyl-1,3,5-hexatriene-p-toluene-sulfonate salt (TMA-DPH). Cells were resuspended in 1 ml of saline and immediately mixed with 1 ml of 1×10^{-6} M TMA-DPH in PBS, pH 7.4.

B. Rat nerve cells

Treatment	DPH FP Arbitrary unit (%)	TMA-DPH FP Arbitrary unit (%)	Cholesterol ($\mu\text{g}/\text{mg}$ protein)
None	0.285 \pm 0.005 § (100)	0.336 \pm 0.007 (100)	157.64 \pm 29 §
PrP106-126	0.329 \pm 0.003 * (115)	0.352 \pm 0.006 * (105)	202.88 \pm 45 *
PrP106-126 (scrambled)	0.288 \pm 0.003 (100)	0.334 \pm 0.005 (100)	132.88 \pm 6

* $p < 0.05$, ** $p < 0.01$, compared with controls; § $p = 0.01$ versus untreated HL60 cells

The FP values were immediately recorded at 25°C. Percent (% , in brackets next to FP values) indicates the FP value of treated cells as a percentage of the basal value. Cellular cholesterol content was determined as described in Materials and Methods. Each value is the mean \pm standard deviation of at least six determinations.

Table 2. Effect of PrP 106-126 on cholesterol uptake and release.

Treatment	Cholesterol uptake (% of total radioactivity)	Cholesterol release (% of total radioactivity)
None	9.9 \pm 3	71 \pm 3
PrP106-126	17.0 \pm 7	10 \pm 2 **
PrP106-126 (scrambled)	28.7 \pm 4	65 \pm 12

For cholesterol uptake experiments, HL60 cells (1×10^6 /ml) were treated at 37°C for 24 h with 50 μM PrP 106-126 or PrP 106-126-scrambled in the presence of 33.5 μM of [^{14}C] cholesterol. Cells were then centrifuged at 550 x g for 10 min, washed twice with PBS, pH 7.4, and the amount of radioactivity taken up was determined in

the pellet. For cholesterol release experiments, HL60 cells (1×10^6 /ml) were incubated with 33.5 μM [^{14}C]cholesterol for 20 h at 37°C. Cells were then centrifuged at 550 x g for 10 min, washed twice with PBS, pH 7.4, resuspended (1×10^6 /ml) and exposed to 50 μM PrP 106-126 or its scrambled mate. After 24 h at 37°C cells were centrifuged at 550 x g for 10 min and the radioactivity released was determined in the supernatant. ** $p < 0.01$ compared with untreated cells.

Table 3. Effect of PrP 106-126 on HMG-CoA reductase activity.

Treatment	HMG-CoA reductase activity in nerve cells ($\text{pmol}/\text{min}/\text{mg}$ protein)	HMG-CoA reductase Activity in HL60 cells ($\text{pmol}/\text{min}/\text{mg}$ protein)
None	23.1 \pm 6.4	13.1 \pm 4
PrP106-126	10.8 \pm 2.9 **	5.0 \pm 1 **
PrP106-126 (scrambled)	17.2 \pm 5.0	11.4 \pm 5

Cells (1×10^6 /ml) were incubated at 37°C for 24 h with 50 μM PrP 106-126 or PrP 106-126-scrambled. At the end of the incubation, nerve cells were detached by trypsinization, centrifuged at 550 x g for 10 min and washed with saline. HL60 cells were centrifuged and washed as above. Cell lysate was then used to determine the activity of HMG-CoA reductase as

described in the Methods. Each value is the mean \pm standard deviation of at least six measurements. ** $p < 0.01$ versus untreated cells.

To investigate whether the rigidifying effect specifically involved the plasma membrane only or the overall cell membranes, we measured the cellular FP value using the plasma membrane-specific probe TMA-DPH. DPH and its cationic analog TMA-DPH have similar spectral characteristics but the charged substituent in TMA-DPH provides a surface anchor, improving its localization in the plasma membrane. TMA-DPH and DPH are not only located in different cellular compartments but also at different depths within the lipid bilayer. This leads to higher FP values for TMA-DPH than for DPH. As shown in Table 1, the basal FP value for TMA-DPH in HL60 cells was higher than in nerve cells. This might be due to changes in membrane surface area or cell volume. In HL60 and nerve cells exposed to 50 μ M PrP 106-126 the TMA-DPH-related FP values were about 8 % and 5 % higher, respectively, indicating that the peptide had less effect on the plasma membrane than on the overall cell membranes (characterized by the FP value increase of 56 % and 15 % for HL60 and nerve cells, respectively, Table 1).

We then set up experiments to determine whether the effect of PrP 106-126 on cell membrane microviscosity was related to the basal membrane lipid composition, particularly to cholesterol content. Nerve cells, which had a higher basal DPH-related FP value (compared with HL60 cells), also had 3.6 times higher cholesterol content (Table 1). To investigate whether PrP 106-126's lower rigidification effect on nerve cells compared with HL60 cells is related to the membrane lipid composition, we enriched HL60 cells with cholesterol (HL60-Chol).¹⁷ This procedure yielded the cells with higher cholesterol content (HL60 cells 40.5 ± 9 μ g cholesterol per mg protein and HL60-Chol cells 64.7 ± 5 μ g cholesterol per mg protein, $p < 0.01$, $n=6$) and higher FP value (HL60 cells 0.237 ± 0.002 and HL60-Chol cells 0.251 ± 0.001 , $p < 0.01$, $n=6$). PrP 106-126 (50 μ M) caused significantly less rigidification ($p < 0.01$, $n=6$) in HL60-Chol cells (FP value 0.321 ± 0.016 , 28 % higher than control no peptide condition) than in HL60 cells (FP value 0.376 ± 0.013 , 56% higher than control), suggesting that the PrP 106-126 peptide ability to cause membrane rigidification is related to the cell cholesterol content.

We then investigated whether PrP 106-126 affected the cellular cholesterol content. As reported in Table 1, 50 μ M of the peptide in 24 h significantly increased the cholesterol content of HL60 and nerve cells (by about 3.4 times and 1.3 times, respectively), whereas PrP 106-126-scrambled had no such effect. Thus, the rigidifying effect of PrP 106-126 could be ascribed both to an inherent property of the peptide to interact

physically with the cell membranes and to its ability to raise the cellular cholesterol content.

To verify whether PrP 106-126 affects the ability of cells to uptake extracellular cholesterol, we incubated HL60 cells for 24 h with 50 μ M of the peptide. We did not observe the increase of cholesterol uptake in the presence of the peptide (Table 1). However, 50 μ M PrP 106-126 significantly inhibited the ability of cells to release cholesterol (by about 86 %) whereas PrP 106-126-scrambled was not effective (Table 2).

We then investigated whether the accumulation of cholesterol affected its' cellular biosynthesis. We measured how 50 μ M PrP 106-126 affected the activity of HMG-CoA reductase, the enzyme catalyzing the first step of cholesterol biosynthesis.²² As reported in Table 3, PrP 106-126 significantly reduced the activity of HMG-CoA reductase, by about 53 % and 60 %, in nerve and HL60 cells, respectively, whereas the scrambled peptide had no such action. Thus the change in cholesterol homeostasis in the examined cells exposed to PrP 106-126 is likely not due to a direct effect of the peptide on sterol synthesis but due to a combination of other biochemical and biophysical alterations (see below).

This study focused on some important biophysical and biochemical effects underlying the action of the prion protein fragment PrP 106-126 on cell membranes. We used nerve cells, sensitive to the chronic toxic action of the peptide, and HL60 cells, which are resistant. After 24 h of incubation, PrP 106-126 was not cytotoxic for either HL60 or nerve cells (100 ± 3 % and 100 ± 5 % of cell viability in HL60 and nerve cells, respectively, $n=8$). Nerve cells had higher basal membrane microviscosity and a higher endogenous cholesterol content. PrP 106-126 had less profound effect on membrane fluidity and cholesterol accumulation in neurons (Table 1); HL60 cells, like other non-neuronal cells,²³ were not sensitive to the peptide's chronic cytotoxicity. In fact, when exposed to 50 μ M PrP106-126 for 7 days HL60 cells viability was not affected (100 ± 2 % and 95 ± 3 %, respectively, $n=6$). The same treatment, however, caused the death of about 37 % of nerve cells (cell viability for untreated and treated cells was 100 ± 3 % and 62 ± 5 %, respectively, $n=6$, $p < 0.01$).²⁴ The above might be due to the fact that non-neural cells can better adapt to the changes in membrane and lipid homeostasis.

Cholesterol turnover in the brain is much slower than in other tissues, with a half-life of about six months.²⁵ Therefore neurons, unlike other cell types, require constant supply of lipid molecules.²⁶ Thus, changes in neuronal membrane homeostasis can cause irreversible response, making them more

susceptible to toxic stimuli and less vulnerable to the repair.

There is increasing evidence that biological misregulation of cholesterol homeostasis plays a key role in the impairment of synaptic plasticity and neuronal degeneration.^{27,28} Moreover, the involvement of lipids in some neurodegenerative disorders was suggested. In particular, disturbances in cholesterol synthesis and homeostasis may be related to several neurological pathologies, such as Alzheimer's and Niemann Pick diseases.^{26,29,30}

The accumulation of cholesterol in cell membranes leads to the redistribution of membrane proteins and membrane traffic.¹⁴ Furthermore, Alzheimer's modifications in the neurochemistry of key proteins and oxidative stress reactions may reflect a transient physiological mechanism serving to compensate the impaired brain cholesterol dynamics and/or associated neurotransmission and synaptic plasticity failure.^{30,31-34}

It has been proposed that prion toxicity results from an interaction with cell membranes^{15,35} leading to changes in membrane viscosity and the formation of ion channels. We propose that the peptide PrP 106-126, similar to the full length prion protein, can interact with the ion transport systems and ion channel formation. Thus, the membrane rigidification induced by this peptide and accompanied by an alteration of cholesterol homeostasis, may affect the traffic of transmembrane proteins, favor the stabilization of ion channels, and contribute to the peptide neurodegenerative effect.

Cholesterol is not uniformly distributed in cell membranes but is condensed in some paths of a high density of cholesterol and sphingolipids, which promote the stability of different membrane proteins. These membrane microdomains, also known as "caveolae", may act as portals to regulate cellular cholesterol homeostasis, and play a role in the structure-specific function of the prion peptides.^{14,31} The sphingomyelin- and cholesterol-rich surface membrane microdomains of the caveolae are important for the insertion and conformational changes of the prion proteins.¹⁴ The increase of cellular cholesterol content caused by PrP 106-126 may cause cholesterol accumulation in caveolae, thus facilitating buildup of the prion peptide and its cytotoxic effects. Interestingly, the role of Alzheimer's amyloid beta protein in cholesterol synthesis, its membrane distribution, and the modulation of membrane lipids via oxidative mechanisms was shown previously^{27,31-34}

We observed that mediated by PrP 106-126 accumulation of cholesterol (via inhibited cellular cholesterol release) decreased *de novo* cholesterol biosynthesis and reduced the activity of HMG-CoA

reductase possibly by a negative feedback mechanism. This enzyme catalyzes the conversion of HMG-CoA to mevalonic acid, affecting the synthesis of mevalonate-derived bioactive sterols and nonsterol metabolites from the cholesterol synthesis pathway.²² Inhibition of HMG-CoA reductase activity may reduce the production of other lipids critical for neuronal function or for prion-membrane interactions.^{27,28,36}

Our data indicate for the first time that PrP 106-126 interacts with cell membranes, and modifies cholesterol homeostasis. These findings should facilitate elucidation of the relationship between cell membrane lipid composition (particularly cholesterol and sphingomyelin) and the progression of prion-related encephalopathies. Future studies, however, are needed to investigate the intracellular distribution of cholesterol and PrP 106-126 at intracellular levels to better understand the molecular mechanisms underlying this effect.

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ABBREVIATION

Abnormal isoform of prion protein (PrPSc), cellular isoform of prion protein (PrPC), 1,6-diphenyl-1,3,5-hexatriene (DPH), fetal bovine serum (FBS), fluorescence polarization (FP), fluorescence polarization oriented parallel (FP1), fluorescence polarization oriented perpendicular (FP2), hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA), HL60 cells enriched in cholesterol (HL60-Chol), membrane microviscosity (η), phosphate buffered saline (PBS), 1-(4-trimethylammoniumphenyl)-6-phenyl-1,3,5-hexatriene-p-toluene-sulfonate salt (TMA-DPH).

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