The Voltage-dependent Anion Channel 1 Mediates Amyloid β Toxicity and Represents a Potential Target for Alzheimer Disease Therapy*

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The voltage-dependent anion channel 1 (VDAC1), found in the mitochondrial outer membrane, forms the main interface between mitochondrial and cellular metabolisms, mediates the passage of a variety of molecules across the mitochondrial outer membrane, and is central to mitochondria-mediated apoptosis. VDAC1 is overexpressed in post-mortem brains of Alzheimer disease (AD) patients. The development and progress of AD are associated with mitochondrial dysfunction resulting from the cytotoxic effects of accumulated amyloid β (Aβ). In this study we demonstrate the involvement of VDAC1 and a VDAC1 N-terminal peptide (VDAC1-N-Ter) in Aβ cell penetration and cell death induction. Aβ directly interacted with VDAC1 and VDAC1-N-Ter, as monitored by VDAC1 channel conductance, surface plasmon resonance, and microscale thermophoresis. Preincubated Aβ interacted with bilayer-reconstituted VDAC1 and increased its conductance ~2-fold. Incubation of cells with Aβ resulted in mitochondria-mediated apoptotic cell death. However, the presence of non-cell-penetrating VDAC1-N-Ter peptide prevented Aβ cellular entry and Aβ-induced mitochondria-mediated apoptosis. Likewise, silencing VDAC1 expression by specific siRNA prevented Aβ entry into the cytosol as well as Aβ-induced toxicity. Finally, the mode of Aβ-mediated action involves detachment of mitochondria-bound hexokinase, induction of VDAC1 oligomerization, and cytochrome c release, a sequence of events leading to apoptosis. As such, we suggest that Aβ-mediated toxicity involves mitochondrial and plasma membrane VDAC1, leading to mitochondrial dysfunction and apoptosis induction. The VDAC1-N-Ter peptide targeting Aβ cytotoxicity is thus a potential new therapeutic strategy for AD treatment.

Alzheimer disease (AD)3 pathology is characterized by cognitive decline, brain synaptic dysfunction, inflammatory responses, and mitochondrial dysfunction (1). The aggregation of the amyloid β peptide (Aβ) is proposed to play a key role in AD pathogenesis (2). Aβ is the post-proteolytic product of sequential cleavages of amyloid precursor protein by β-secretase and γ-secretase. The commonly used 42-amino acid-long Aβ (Aβ42) self-aggregates into oligomers, with larger aggregates forming Aβ plaques. However, soluble Aβ oligomers were proposed to be the cytotoxic form of the protein, acting extracellularly (3).

The severity of dementia and brain hypometabolism has been previously shown to be tightly linked (4), with brain hypometabolism preceding clinical signs of AD. This is directly associated with mitochondrial dysfunction being an early event in AD pathogenesis, as reflected in reduced metabolism, disruption of Ca2+ homeostasis, increased free radical production, and lipid peroxidation (5–9). Aβ also affects mitochondrial respiration (10) and activates cytochrome c release, resulting in apoptosis (11). Importantly, Aβ does not cause toxicity in cells depleted of mitochondria (12). Finally, the mitochondrial protein, the voltage-dependent anion channel (VDAC), was shown to participate in Aβ-induced toxicity (13, 14).

VDAC1 transports ions, Ca2+, cholesterol, and metabolites across the outer mitochondrial membrane and participates in the release of mitochondrial pro-apoptotic proteins to the cytosol and interacts with apoptosis regulatory proteins (15, 16). Thus VDAC1 appears to be a convergence point for a variety of cell survival and death signals.

VDAC1 is a β-barrel protein with a 25-residue-long N-terminal domain lying inside the pore but able to exit the pore, with its mobility-controlling channel gating and interaction with anti-apoptotic proteins (16–20). Moreover, cells expressing N-terminal segment-truncated VDAC1 are apoptosis-resistant (19). These findings indicate that the N-terminal domain is required for apoptosis induction.

High levels of VDAC1 were demonstrated in the dystrophic neurites of AD brains and amyloid precursor protein transgenic mice (21). Aβ–VDAC interactions are toxic to AD-affected neurons (22). VDAC1 interacts with Aβ and phosphorylated Tau, leading to mitochondrial dysfunction.

mVDAC1, murine VDAC1; Cyto c, cytochrome c; EGS, ethylene glycol bis[succinimidylsuccinate]; PLB, planar lipid bilayer; MST, microscale thermophoresis; SPR, surface plasmon resonance; N-Ter, N-terminal; HK, hexokinase; ROS, reactive oxygen species.

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tion (14). Finally, an increase in nitrated VDAC1 in AD, reflecting oxidative damage to VDAC, was reported (23), possibly affecting cell energy and metabolites homeostasis (24).

VDAC1 was shown to be localized to the plasma membrane of various cells, including the brain post-synaptic membrane fraction (25). The involvement of plasmaemal VDAC (pVDAC) in AD was proposed (13, 26), serving as an “amyloid-regulated” channel involved in apoptosis (27).

Here, we demonstrate VDAC1 involvement in Aβ entry into the cell and in Aβ-mediated apoptosis. By measuring VDAC1 conductance and using SPR methodology, we show that Aβ directly interacts with VDAC1, specifically with its N-terminal region. Moreover, VDAC1-N-terminal peptides prevented Aβ cell penetration and its pro-apoptotic activity. Aβ cell penetration and toxicity were prevented in cells depleted of VDAC1 using siRNA. A similar effect was recorded in cells in which Aβ-VDAC1 interaction was inhibited by VDAC1 N-terminal peptides. These findings point to VDAC1 as a target for novel therapeutic strategies for AD treatment.

**Experimental Procedures**

**Materials**—Dimethyl sulfoxide (DMSO), EDTA, EGTA, leupeptin, phenylmethylsulfonyl fluoride (PMSF), propidium iodide, and sodium selenite were purchased from Sigma. siRNA was synthesized by Dharmaco (Lafayette, CO) or obtained from Genepharma (Suzhou, China). JetPRIME was from PolyPlus Transfection (Illkirch, France). Ethylene glycol bis(succinimidylsuccinate) (EGS) was obtained from Pierce. 4’,6-Diamidino-2-phenylindole (DAPI), Cy3-conjugated anti-rabbit antibodies, anti-Aβ (ab2539), and anti-VDAC1 antibodies directed against the N-terminal region of VDAC1 (anti-VDAC1 ab135585) were purchased from Abcam (Cambridge, England). HRP-conjugated anti-rabbit antibodies were from Invitrogen. Dulbecco’s modified Eagle’s medium (DMEM), fetal calf serum, L-glutamine, and penicillin-streptomycin solution were purchased from Biological Industries (Beit Haemek, Israel). Cellusports peptide arrays were obtained from INTAVIS Bioanalytical Instruments (Kln, Germany).

**Peptides**—Amyloid β (Aβ residues 1–42) and VDAC1 N-terminal (1MAVPPPTYADLKSARDVTGKYGFGL26) peptides were synthesized by GL Biochem (Shanghai, China). The peptides were dissolved in DMSO and stored as a 2 mM solution in 10–20% DMSO at –80 °C until use. To form Aβ oligomers, Aβ was preincubated (2 mM) at 37 °C for 2–7 days before use. Aβ was also dissolved in hexafluoro-2-propanol and evaporated using nitrogen gas and stored at –80 °C until use. For oligomeric Aβ formation, dried Aβ was dissolved in DMSO as a 5 mM solution and diluted with PBS to 0.2 mM (28). The final concentration of DMSO in untreated and Aβ-treated cells was 0.5% or less.

**Cell Lines**—PC12 (rat neuroblastoma), SH-SY5Y (human neuroblastoma), and HeLa (human cervical) cells were grown in DMEM, supplemented with 10% fetal calf serum, 2 mM L-glutamine, 100 units/ml penicillin, 100 µg/ml streptomycin, 1 mM sodium pyruvate, and nonessential amino acids, and maintained in a humidified atmosphere at 37 °C with 5% CO2.

**Plasmids and Cell Transfection**—Logarithmically growing SH-SY5Y cells were transiently transfected with the desired plasmid using the JetPRIME transfection agent according to the manufacturer’s instructions. The transfect constructs corresponded to plasmid pEGFP encoding for HK-I-GFP under control of the CMV promoter or the hTERT promoter to drive the expression of murine VDAC1 (mVDAC1)-GFP-CAAX or mVDAC1-CAAX. The CAAX motif corresponds to the cysteine-valine-isoleucine-methionine (CVIM) sequence of K-Ras and VDAC1, which may regulate cell entry into apoptosis (27).

**Electron Microscope and Negative Staining**—Preincubated Aβ aliquots (20 µM) were mounted on 300-knob copper Formvar/carbon grids. The grids were washed with double-distilled water and stained with 1% phosphotungstic acid, pH 7.25, for 1 min. Samples were viewed in a JEOLEM 1230. (JEOL, Japan) transmission electron microscope. Digital images were collected with a Gatan model 830 ORIUS SC200 CCD camera using Gatan Digital Micrograph (DM) software.

**Cell Treatment with Aβ and VDAC1-N-Ter Peptides**—PC12 or SH-SY5Y cells were seeded in 96-well plates (1 × 104 cells/well) or 6-well plates (2 × 105 cells/well), and 24 h later the cells were treated with different concentrations of the peptides (Aβ and/or VDAC1-N-Ter) in serum-free DMEM medium. After 6–48 h of incubation, the cells were analyzed as described below.

**XTT Cell Survival Assay**—Cells either untreated or treated with Aβ (2–30 µM) and VDAC1 N-Ter peptide (1–30 µM) were analyzed for cell viability using XTT according to the manufacturer’s protocol, except that cells were washed twice with PBS before the addition of the XTT solution.

**Cell Death Analysis**—The death of Aβ-treated and untreated cells was analyzed by acridine orange and ethidium bromide staining (30) and confocal microscopy (Olympus IX81). In each independent experiment ~300 live early and late apoptotic cells were counted. Apoptotic cell death was also analyzed by propidium iodide staining using a flow cytometer (BD Biosciences) and FACsEclipse flow cytometer ec800 software (Fourtec).

**Immunocytochemistry-Confocal Microscopy**—Cells were grown on coverslips and after treatment were fixed for 15 min with 4% paraformaldehyde prepared in PBS, washed with PBS, and incubated 5 min with 0.3% Triton X-100 in PBS and then with anti-Aβ antibodies (1:400) and anti-Cy3 antibodies (1:400) for 2 h and for 1 h with Cy3-conjugated anti-rabbit (1:300) and Cy2-conjugated anti-mouse (1:300) secondary antibodies. The cells were then stained with DAPI (0.07 µg/ml) and viewed in an Olympus IX81 confocal microscope. For HK-I-GFP imaging, cells grown on coverslips were transfected with plasmid pEGFP-HK-I-GFP and 24 h later were treated with Aβ, fixed with paraformaldehyde (4%), rinsed for 30 min with PBS, stained with DAPI (0.07 µg/ml) and mounted and visualized by confocal microscopy.

**VDAC1 Silencing by siRNA Transfection**—For silencing VDAC1, human-specific siRNA (sense, 5’-23ARACACUGGCACCCGAGAUUA246-3’; antisense, 5’-UAUAUCUGGUGCCGACAGUGU3’; VDAC1-siRNA) or scrambled siRNA (sense, 5’-GCAAACUCCCAAGAGGUUA3’; antisense, 5’-AUCAC-
VDAC1 Mediates Aβ Cytotoxicity

CUCUGGAUGUUUGC-3’) (Scr-siRNA) were used. 2’-O-Methyl-modified nucleotides are indicated in bold and underlined. SH-SY5Y cells were seeded in 12-well plates (3 × 10⁴ cells/well) and 24 h later were transfected with human VDAC1-siRNA (50 nm) (Drhmacon) using jet-Prime transfection reagent according to the manufacturer’s instructions. After 24 h the cells were left untreated or treated with Aβ for the indicated times and subjected to the desired assay.

Cell Lysate Preparation—Cells were harvested, washed twice with PBS, and resuspended in buffer A (20 mM HEPES, pH 7.4, 150 mM NaCl, 1 mM EDTA 1 mM EGTA, 10% glycerol, and 1 mM MgCl₂). Samples were then sonicated for 10 s and centrifuged for 10 min at 600 × g, and the supernatant was collected. Protein concentration in the sample was determined using the Bradford method. For electrophoresis, samples were diluted 4:1 with a 4× sample buffer containing 40% glycerol, 4% β-mercaptoethanol, 8% SDS, 0.26 M Tris, pH 6.8, and bromophenol blue and incubated for 10 min at 70 °C. Aliquots (15–30 μg) were subjected to SDS-PAGE.

Chemical Cross-linking—HeLa and SH-SY5Y cells incubated with and without Aβ were harvested and incubated (1.5–3 mg/ml) with the cross-linking reagent EGS (150–300 μM) in PBS, pH 8.2, for 15 min at 30 °C. Samples (60–90 μg) were subjected to SDS-PAGE and immunoblotting using anti-VDAC1 antibodies as described below. Quantitative analysis of immuno reactive VDAC1 dimer bands was performed using Image Gauge software provided by Fujifilm.

Immunoblot Analysis—After SDS-PAGE and electrotransfer to nitrocellulose membranes, proteins were immunostained by incubation in blocking solution containing 5% nonfat dry milk and 0.1% Tween 20 in Tris-buffered saline followed by incubation with anti-VDAC1 (1:10,000) or anti-actin (1:20,000) antibodies. The membranes were then incubated with HRP-conjugated anti-rabbit IgG (1:15,000) as secondary antibodies. Antibody binding was revealed using an enhanced chemiluminescence (ECL) assay from Biological Industries (Beit Haemek, Israel) for detection of HRP activity. Densitometric quantification of band intensity was performed using Image Gauge software provided software (Fujifilm) with values normalized to the intensities of β-actin.

VDAC1 Reconstitution into a Planar Lipid Bilayer and Analysis of Channel Activity—VDAC1 purified from rat liver mitochondria was used for channel reconstitution into a planar lipid bilayer (PLB) prepared from soybean aseloctin dissolved in n-decane (30 mg/ml) (18). Purified VDAC1 was added to the cis chamber containing 0.5 mM NaCl and 10 mM Hepes, pH 7.4. After one or more channels were inserted into the PLB, excess protein was removed by perfusing the cis chamber to prevent further channel incorporation. Currents were recorded by voltage-clamping using a Bilayer Clamp BC-525B amplifier (Warner Instruments, Hamden, CT). Current was measured with respect to the trans side of the membrane (ground). The currents were low pass-filtered at 1 kHz and digitized on-line using a Digidata 1440-interface board and Clampex 10.2 software (Axon Instruments, Union City, CA).

Microscale Thermophoresis (MST)—Purified VDAC1 was fluorescently labeled using the NanoTemper blue protein-labeling kit. Fluorescently labeled VDAC1 (100 nm) was incubated with different concentrations of Aβ (1.2–100 μM) in PBS buffer. After 20 min of incubation, 3–5-μl aliquots were loaded into MST-grade glass capillaries (NanoTemper Technologies), and thermophoresis was measured with a NanoTemper Monolith-NT115 system (20% light-emitting diode, 40% IR laser power). MST was also conducted with a NanoTemper Monolith NT Label-Free system. In this case the intrinsic fluorescence of a protein derived from its tryptophan content was used to follow the interaction of Aβ (1.2–100 μM) with the nonlabeled VDAC1 (100 nm). After 20 min of incubation, 3–5-μl aliquots were loaded into MST-grade glass capillaries, and thermophoresis was measured (10% UV light-emitting diode, 20% IR laser power).

Surface Plasmon Resonance (SPR)—SPR measurements collected using the ProteOn-XPR36 system (Bio-Rad) were used to study the interaction of the VDAC1 N-Ter peptide with Aβ peptide immobilized on a ProteOn GLC Sensor Chip (Bio-Rad) according to the manufacturer’s instructions. The VDAC1 N-Ter peptide was diluted in running buffer (150 mM NaCl, 0.005% Tween 20, 4% (v/v) DMSO, 10 mM Pı, pH 7.4) and injected onto the sensor chip at varying concentrations at a flow rate of 40 μl/min at 25 °C. Responses (resonance units (RU)) were monitored using the ProteOn imaging system and related software tools. Dose-response curves of Aβ binding to the N-Ter peptide were obtained using the steady-state values. The apparent binding affinities (K_a) of the peptides were derived from Hill plot fits.

Statistics—Means ± S.E. of results obtained from independent experiments were calculated. Alternatively, due to the known high variability in the degree of cell death induced by Aβ treatment, a representative result is presented.

Results

Aβ Directly Interacts with VDAC1—To demonstrate the interaction of Aβ with VDAC1, the effect of Aβ on VDAC1 channel conductance was studied (Fig. 1, A–D). Purified mitochondrial VDAC1 was reconstituted into a PLB, and channel conductance was monitored under voltage-clamp conditions. Aβ peptide, non-incubated or preincubated for 5 days to allow oligomeric structures to form, directly interacted with PLB-reconstituted VDAC1 to alter channel conductance. Although preincubated Aβ enhanced VDAC1 channel conductance (Fig. 1, A and B), non-incubated Aβ decreased channel conductance (Fig. 1, C and D). VDAC1 channel conductance was voltage-dependent, with the highest conductance obtained between −20 and +20 mV and with decreased conductance seen at both high negative or positive potentials (Fig. 1, B and D). Preincubated Aβ enhanced VDAC1 conductance at all applied voltages (Fig. 1B), whereas non-incubated Aβ decreased channel conductance at all voltages. The increase in VDAC1 conductance in the presence of preincubated Aβ suggested that additional VDAC1 molecules were recruited to form an oligomeric structure with a large pore size, possibly as hetero-oligomers with Aβ, hence resulting in higher conductance. Non-incubated Aβ most probably decreased channel conductance via interaction with VDAC1 and/or blocking the channel pore. It has been shown that at relatively high concentrations (>50 μM) Aβ increases lipid bilayer conductance (49). However, Aβ
VDAC1 Mediates Aβ Cytotoxicity

A. Pre-incubated Aβ

B. Voltage sensitivity of Aβ conductance at a given voltage was normalized to the conductance at 10 mV. The effect of voltage from 60 mV to 60 mV was tested. The average steady-state conductance of voltage from 60 mV to 60 mV was tested. The average steady-state conductance at a given voltage was normalized to the conductance at 10 mV. The recordings were taken before (●) and 5 min after (○) the addition of Aβ (2 µM) incubated 5 days at 37 °C. A representative of three independent experiments is shown. B, the effect of Aβ (incubated 5 days at 37 °C) on VDAC1 conductance as a function of voltage from 60 mV to 60 mV was tested. The average steady-state conductance at a given voltage was normalized to the conductance at 10 mV. The recordings were taken before (●) and 5 min after (○) the addition of Aβ (2 µM), similar experiment as in A, except that non-incubated Aβ was used. D, similar experiment as in B, except that non-incubated Aβ was used. E. The effect of Aβ (2 µM) (incubated for 5 days at 37 °C) on the conductance of PLB, in response to voltage step from 0 to 10 mV, was tested with the currents recorded before and 15 min after the addition of Aβ. A representative of three independent experiments is shown. F, purified VDAC1 was fluorescently labeled using the NanoTemper blue protein-labeling kit according to the manufacturer’s instructions. VDAC1 (100 µM) was incubated for 20 min with Aβ (1.2–100 µM), and thermophoresis was measured using the MonolithNT115 apparatus. A Kd of 50 µM was determined. A representative of three independent experiments is shown. G, change in normalized fluorescence (ΔF Norm) as a function of Aβ concentration (Fig. 1, F and G). A curve fitted to the data revealed dissociation constants of 16.6 and 50 µM for labeled and non-labeled VDAC1, respectively, considering monomeric Aβ as the interacting molecule. Taken together, these data show that Aβ can interact with VDAC1 and increase its conductance, conceivably by forming VDAC1-Aβ hetero-oligomers.

VDAC1-derived Peptides Interact with Aβ—As the N-terminal of VDAC1 is known to be the interacting part of the protein (32), we decided to examine whether the VDAC1-N-Ter peptide also interacted with Aβ. We tested the direct interaction of the VDAC1 N-Ter peptide with Aβ using SPR technology. Aβ peptide was coupled to an SPR biosensor, the ProteOn GLC chip, and increasing concentrations (0–400 µM) of the N-Ter peptide were injected onto the sensor chip, allowing for its binding to immobilized Aβ to be monitored. The VDAC1 N-Ter peptide bound to immobilized Aβ in a concentration- and time-dependent manner (Fig. 2, A and B). The best fit for VDAC1 N-Ter peptide binding to Aβ when analyzed by Hill plot analysis revealed two association constants (Kd) of 2.5 and 900 µM (Fig. 2B), suggesting interaction with 2 or more binding sites. It should be noted that these Kd values were calculated based on monomeric Aβ concentration even though preincubated Aβ is found in the oligomeric form (data not shown). As such, these apparent Kd values do not reflect actual binding affinities. This result, however, underscored the N-terminal regulatory portion of VDAC1 as being the relevant moiety in the Aβ-VDAC1 interaction.

Preincubated Oligomeric Aβ Is the Cytotoxic Form of the Protein—To better characterize the Aβ oligomeric state, we analyzed the structural organization of non-incubated and preincubated Aβ using negative staining electron microscopy. Preincubation of Aβ for 5–7 days resulted in the formation of fibrils of oligomeric Aβ, as compared with non-incubated Aβ (Fig. 3, A and B).

The effects of non- and preincubated Aβ on cell viability were analyzed using XTT. Treating rat neuroblastoma PC12 cells for 48 h with varying concentrations of non-incubated Aβ had little or no effect on cell viability. On the other hand, preincubated Aβ significantly decreased cell viability (Fig. 3C). Thus, as shown previously (33), preincubated Aβ with its oligomeric structure is the cytotoxic form.

VDAC1-N-Ter Peptide Prevents Aβ Cell Penetration and Aβ-induced Apoptotic Cell Death—As we found that VDAC1 (Fig. 1) and, more specifically, its N-terminal domain (Fig. 2) interacted with Aβ, we next tested the effect of the VDAC1 N-terminal non-cell-penetrating peptides on Aβ-mediated cell toxicity (Fig. 4). PC12 cells were treated with varying concentrations of Aβ in the presence or absence of VDAC1 N-Ter peptide. Strikingly, Aβ toxicity was prevented when incubated alone, at the concentrations used in our experiments (2.5 µM), had no effect on the conductance of the bilayer, implying that low concentrations of Aβ do not form current-conducting channels in the PLB (Fig. 1E).

To further demonstrate the VDAC1-Aβ interaction, we employed MST, an approach that enabled us to obtain quantitative insight into the interaction of VDAC1 with preincubated Aβ and extract a binding affinity coefficient (Kd) (31). In MST, the binding of a labeled version of the molecule of study to an interacting partner influences the thermal migration behavior of that labeled protein. The depletion of fluorescence at the spot in the protein solution where heat is applied is measured as a function of increasing interacting partner concentration, allowing for the calculation of Kd values. Using MST, we analyzed the interaction of Aβ with fluorescently labeled (Fig. 1F) or non-labeled (Fig. 1G) VDAC1. For such studies, purified VDAC1 was labeled with NT-495 (or used unlabeled) and incubated with Aβ peptide (0–100 µM) before MST analysis. The data obtained are presented as the change in normalized fluorescence (ΔF Norm) as a function of Aβ concentration (Fig. 1, F and G). A curve fitted to the data revealed dissociation constants of 16.6 and 50 µM for labeled and non-labeled VDAC1, respectively, considering monomeric Aβ as the interacting molecule. Taken together, these data show that Aβ can interact with VDAC1 and increase its conductance, conceivably by forming VDAC1-Aβ hetero-oligomers.
VDAC1 Mediates Aβ Cytotoxicity

FIGURE 2. Aβ interacts with VDAC1-based peptides. A, VDAC1 N-Ter peptide interaction with Aβ, as analyzed by SPR. Aβ, immobilized onto a GLC sensor surface, was exposed to the VDAC1 N-Ter peptide (0–400 μM), and the interaction was monitored using the ProteOn imaging system and related software tools. The two lines of each color represent the obtained results and fitted curves. B, Aβ interaction with the VDAC1 N-Ter peptide as analyzed using the heterogeneous ligand interaction model, where one analyte binds two different ligand species, yielding a $K_{d1}$ value of 2.5 μM and a $K_{d2}$ value of 900 μM. RU, response units.

A Non-incubated Aβ  B Pre-incubated Aβ

FIGURE 3. Preincubation of Aβ results in cytotoxic Aβ oligomers. A and B, Aβ (2 μM) was non-incubated (A) or preincubated (B) for 7 days at 37°C. Samples were then diluted to a concentration of 20 μM, and 5 μl aliquots were mounted onto copper grids, stained with phosphotungstic acid as described under “Experimental Procedures,” and viewed in a transmission electron microscope. Scale bar = 100 nm. C, PC12 cells were incubated for 48 h with the indicated concentrations of Aβ preincubated for 7 days at 37°C (●) or with non-incubated Aβ (○). Cell viability was then analyzed using the XTT assay.

Together with the peptide (Fig. 4A), VDAC1 N-Ter peptide-mediated protection was dose-dependent (Fig. 4B), preventing Aβ-induced cell death when present even at a molar ratio of 1:6 (VDAC1 N-Ter: Aβ), suggesting that the N-terminal peptide-mediated effect did not require binding of one N-Ter peptide to each Aβ molecule.

To further demonstrate Aβ-induced apoptotic cell death and the protective effect of the VDAC1 N-Ter peptide, SH-SY5Y human neuroblastoma cells were treated with Aβ in the presence or absence of the peptide, and apoptotic cell death was analyzed using acridine orange and ethidium bromide staining (Fig. 4C). Aβ-induced apoptotic cell death, as reflected in membrane blebbing (Fig. 4C, arrows) and cell nuclear staining with both acridine orange and ethidium bromide (Fig. 4C, arrowheads, orange color), representing the late apoptotic stage, was observed. Cell incubation with the VDAC1 N-Ter peptide dramatically diminished Aβ-induced cell death. Quantitative analysis of the data indicated that Aβ-induced late apoptotic death in ~55% that of the cells (early stage apoptotic cells were not counted). Such death was completely prevented when the cells were incubated with Aβ in the presence of the VDAC1 N-Ter peptide (Fig. 4D). Taken together, these results suggest that VDAC1 N-Ter peptide can prevent Aβ-induced apoptosis in neuronal cells by preventing Aβ cellular entry.

Aβ Entry into SH-SY5Y Cells Is Inhibited by the VDAC1 N-Ter Peptide and by Silencing VDAC1 Expression Using Specific siRNA—To define the possible mechanism by which the VDAC1 N-Ter peptide interferes with Aβ toxicity, SH-SY5Y cells were treated with Aβ in the absence or presence of the peptide and subjected to immunocytochemical staining using anti-Aβ antibodies. The results demonstrated that Aβ immuno-staining is associated with the cell surface as well as seen within the cell (Fig. 5), suggesting Aβ penetration into the cell. Moreover, Aβ was associated with the mitochondria, as anti-Aβ and anti-Cyto c immunostaining overlapped (Fig. 5), pointing to their co-localization (Fig. 5, enlargements). The presence of the VDAC1 N-Ter peptide during Aβ treatment dramatically decreased Aβ-related staining. No co-localization with Cyto c was observed, suggesting that interference with Aβ entry into the cell had occurred (Fig. 5). Because the peptide used does not penetrate the cells, these results suggest that VDAC1 in the cell membrane is involved in Aβ cell penetration and toxicity.

To further explore VDAC1 involvement in Aβ neurotoxicity and cell binding/penetration, VDAC1 expression in SH-SY5Y cells was silenced using siRNA specific to human VDAC1 (Fig. 6A). VDAC1 expression levels, as analyzed by Western blotting, were greatly reduced (74%) after such treatment (Fig. 6A, inset). Comparison of Aβ-induced cell toxicity in VDAC1-expressing and VDAC1-depleted SH-SY5Y cells clearly demonstrated that cells silenced for VDAC1 expression were less sensitive to an
VDAC1 Mediates Aβ Cytotoxicity

Aβ-mediated toxic effect (Fig. 6A). Similarly, VDAC1 depletion protected against Aβ-induced cell death, as analyzed by propidium iodide staining and FACS analysis (Fig. 6B). These findings are in agreement with absence of Aβ from the cell interior (Fig. 6C).

Immunocytochemical staining using anti-Aβ antibodies demonstrated the association of Aβ with the cell membrane and its presence in the cell interior. Aβ, moreover, co-localized with mitochondria, as revealed by the overlap of signals associated with Aβ and Cyto c (Fig. 6C). The presence of intracellular Aβ was strongly decreased in cells depleted of VDAC1 using siRNA (Fig. 6C). An enlargement and merger of the marked cells clearly showed cellular localization of Aβ in cells expressing VDAC1 but not in cells depleted of VDAC1 (Fig. 6C). These results further support the suggestion that VDAC1 is required for Aβ cell penetration and toxicity.

VDAC1 Targeted to the Plasma Membrane and Sensitivity to Aβ—The results obtained with VDAC1-siRNA and VDAC1-N-Ter peptide raise the possibility that Aβ interacts with and enters the cell via plasma membrane-localized or plasmalemmal VDAC1, as previously proposed (34). Indeed, not only mitochondrial VDAC1, but also plVDAC1, was proposed to be involved in AD (34). plVDAC1 is thought to contain a 13-amino acid extension at the N terminus resembling plasma membrane targeting sequences (35). It is not clear, however, if the extension remains with the protein after it is targeted to the plasma membrane. Indeed, plasma membrane-bound VDAC1 was found in various cells, including the post-synaptic membrane fraction from brain (25), and in caveolae and caveolae-like domains (26). Still, this form of the protein remains a matter of dispute (36). The involvement of plVDAC1 in AD was proposed based on VDAC1 being abundant in caveolae and prominent in the dystrophic neurites of senile plaques (26) as well as because of the inhibition of Aβ-induced apoptosis by anti-VDAC antibodies (37).

To explore the effect of plVDAC1 on Aβ cytotoxicity, plasma membrane-targeted VDAC1 was constructed and expressed. Initially, we generated plVDAC1 containing the 13-amino acid-long (MCSFFLVLLLWQNM) extension but found it to also localize to mitochondria (data not shown), making it difficult to visualize the plasma membrane localization of the protein and verify how this affects cell sensitivity to Aβ toxicity. We, therefore, generated VDAC1-GFP-CAAX, predominantly targeting mVDAC1 to the plasma membrane (Fig. 7A). CAAX is a motif previously shown to target proteins to the plasma membrane (38). The CAAX sequence used here comprised cysteine, valine, isoleucine, and methionine. The CAAX box undergoes post-translational prenylation in which either farnesyl (15 carbon) or geranylgeranyl (20 carbon) isoprenoids are added to the cysteine residue of the CAAX box, thus anchoring motif-bearing proteins to the plasma membrane (39). Accordingly, SH-SY5Y cells were transfected to express either VDAC1-GFP-CAAX, and the cellular distribution was analyzed (Fig. 7, A and B), or VDAC1-CAAX, and the sensitivities of the transformed cells to Aβ were examined (Fig. 7, C-F). SH-SY5Y cells transfected to express VDAC1-GFP-CAAX displayed predominantly plasma membrane expression of the chimera, as visualized by confocal microscopy (Fig. 7, A and B).

Next, we expressed VDAC1-CAAX and tested its effect on Aβ cytotoxicity. Immunoblotting analysis clearly demonstrated the expression of VDAC1-CAAX, which appeared as an
antibody-stained band with lower electrophoretic mobility than endogenous VDAC1 (Fig. 7C). As shown previously for mitochondrially expressed VDAC1 (40–45), overexpression of VDAC1-C\textsubscript{AAX} induced cell death (Fig. 7, D–F). Cells incubated with A\textsubscript{2}H9252 underwent cell death, and this was increased in cells expressing VDAC1-C\textsubscript{AAX}, albeit not in an additive or synergistic manner (Fig. 7, D–F). This suggests that cell death induced by A\textsubscript{2}H9252 and by VDAC1-C\textsubscript{AAX} expression involves a similar mechanism of apoptosis induction (40–45) (34).

A\textsubscript{2}H9252 Induces Mitochondrial-bound HK-I Detachment, VDAC1 Oligomerization, and Cytochrome c Release, Leading to Apoptosis—To further elucidate the mode of A\textsubscript{2}H9252 action in apoptosis induction, we examined the effects of A\textsubscript{2}H9252 on several processes associated with apoptosis induction, namely detachment of mitochondrial-bound hexokinase (HK-I), induction of VDAC1 oligomerization, and cytochrome c release.

HK-I was previously shown to bind the mitochondria via VDAC1 (46) and to protect against cell death (29) with its detachment encouraging apoptosis. To examine whether A\textsubscript{β}-induced detachment of HK-I from mitochondria, cells were transfected to express HK-I-GFP and subjected to A\textsubscript{β} treatment. In control cells, HK-I-GFP was clearly localized to the mitochondria, as indicated by the punctuated distribution of fluorescence (Fig. 8Aa, arrows). By contrast, HK-I-GFP fluorescence was diffuse in the A\textsubscript{β}-treated cells, reflecting displacement of HK-I from the mitochondria (Fig. 8A, b and c, arrowheads). Moreover, in A\textsubscript{β}-treated cells, fragmented nuclei were detected (Fig. 8Ac, red arrows). Such fragmentation is a hallmark of apoptotic cell death.

As VDAC1 oligomerization is coupled to apoptosis induced by various apoptosis stimuli (47, 48), we next tested the effect of A\textsubscript{β} on VDAC1 oligomerization in SH-SY5Y and HeLa cells (Fig. 8, B and C). VDAC1 oligomerization was examined after chemical cross-linking with EGS and Western blotting using anti-VDAC1 antibodies. Control cells and cells incubated with A\textsubscript{β} subjected to EGS cross-linking showed the formation of VDAC1 cross-linked products comprising dimers to higher molecular mass complexes (Fig. 8, B and C). VDAC1 oligomerization was highly enhanced upon A\textsubscript{β} treatment, with quantitative analysis of the dimers revealing an increase of 2–2.7-fold in VDAC1 dimer levels in cell exposed to 10\textmu M A\textsubscript{β} (Fig. 8, B and C).

The activity of A\textsubscript{β} in inducing Cyto c release from the mitochondria, a key step in apoptosis induction, was analyzed in SH-SY5Y cells by Western blot analysis of the cytosolic fraction of cells treated with the A\textsubscript{β} peptide (Fig. 8D). In cells treated with A\textsubscript{β}, Cyto c was detected in the cytosolic fraction, in contrast to control cells not treated with A\textsubscript{β}. Immunostaining with anti-ATP synthase 5A antibodies confirmed the efficiency of separation between the mitochondrial and cytosolic fractions. A\textsubscript{β}-induced Cyto c release was further demonstrated by immunocytochemistry using anti-Cyto c antibodies. Representative confocal images of control cells showed that Cyto c is located within the mitochondria (Fig. 8Ea). The staining, however, was
significantly weaker in cells incubated with Aβ/H9252 (Fig. 8E), reflecting Cyto c release from mitochondria to the cytosol and probable degradation of the protein. These data support the suggestion that intracellular Aβ-mediated neuronal cytotoxicity is generated by HK-I detachment and VDAC1 oligomerization, eventually leading to Cyto c release and apoptosis.

Discussion

In this study we presented results that contribute to the better understanding of oligomeric Aβ-mediated toxicity and delineate the involvement of VDAC1 in Aβ cell penetration and toxicity while pointing to the VDAC1-N-terminal domain as the Aβ-interacting site and its potential use in preventing Aβ cell toxicity. The results demonstrate that the Aβ toxicity is mediated via its VDAC1-dependent cell penetration and activation of a VDAC1-regulated mitochondrial apoptotic pathway (Fig. 9).

Aβ Interacts with VDAC1 to Mediate Its Cytotoxicity—The direct interaction of Aβ with VDAC1 was clearly demonstrated using the microscale thermophoresis method and by Aβ greatly increasing bilayer-reconstituted purified VDAC1 channel conductance. Because at the low concentrations of Aβ used in this

FIGURE 6. Cells with low levels of VDAC1 show decreased Aβ cell penetration and cytotoxicity. SH-SYSY cells were transfected with VDAC1-siRNA (50 nM) or not transfected, and 24 h later the cells were incubated with 20 µM Aβ for 48 h. A and B, SH-SYSY cells transfected with 50 nM of scrambled (Scr) siRNA or VDAC1-siRNA, and 24 h post-transfection cells were treated with different concentrations of Aβ for 48 h. A, cell viability was assessed by the XTT assay for (Scr) siRNA (●) or VDAC1-siRNA (○)-treated cells. The data represent the mean ± S.D. (n = 3). The inset shows Western blot analysis of VDAC1 protein levels in SH-SYSY cells transfected with Scr- or VDAC1-siRNA. RU indicates relative units, showing a decrease of 74% in VDAC1 level in cells treated with VDAC1-siRNA. B, a similar experiment as in A was carried out, and cell death rate was determined using propidium iodide staining for Scr-siRNA (black) or VDAC1-siRNA (gray)-treated cells. C, cells were fixed and incubated with anti-Aβ antibodies followed by secondary Cy3-conjugated anti-rabbit antibodies (shown in red) and anti-Cyto c antibodies followed by secondary Cy2 conjugated anti-mouse antibodies (shown in green). Cells were also stained with DAPI (blue) and visualized by confocal microscopy. The third and fifth columns represent enlargements of the marked areas. Bar = 10 µm. The lack of Aβ staining in siRNA-treated cells may indicate the Aβ inability of to cross the cell membrane.
study (2.5 μM) there was no effect on bilayer conductance, the Aβ-mediated increase in channel conductance is a result of Aβ modifying VDAC1 conductance. The two-fold increase in channel conductance could thus be a result of increased VDAC1 pore size (Fig. 9). Such an increase in VDAC1 pore size could be obtained when Aβ is inserted into the VDAC1 β-barrel (50) or by Aβ oligomers inducing VDAC1 oligomerization while inserted between the VDAC1 monomers within the VDAC1 oligomeric structure (Fig. 9). Further studies are required to explore the mechanism by which Aβ increases VDAC1 channel conductance. We showed that VDAC1 N-Ter peptide directly interacts with Aβ, preventing both Aβ cell penetration and Aβ cytotoxicity, suggesting that Aβ interacts with the VDAC1 N-terminal domain to mediate its action.

Simulation models defining interaction sites of the Aβ peptide with the N-Ter peptide suggest three possible key interaction sites, namely via the GXXXG (glycine-rich domain) motif present in both peptides, hydrophobic sites and/or charged sites (Fig. 9, A and B). In this first model, the GXXXG motif in the VDAC1 N-Ter peptide is proposed to interact with one of the three GXXXG motifs in the Aβ peptide (Fig. 9A). Indeed, the GXXXG motif in the N-terminal region of pLVADAC1 was previously proposed to interact with the GXXXG motifs of Aβ (34). The GXXXG motif has been linked with dimerization in proteins such as glycophorin (51), human carbonic anhydrase (52), yeast ATP synthase (53), and carnitine palmitoyltransferase (54). The first amino acids of the VDAC1 N-terminal domain are hydrophobic in nature and correspond to additional residues presumed to provide an additional point of contact with Aβ (Fig. 9A). The Aβ peptide and Aβ oligomers present a large hydrophobic volume that may interact with the N-terminal region of VDAC1 (Fig. 9B) and then further intercalate between VDAC1 and the membrane (Fig. 9C). Alternatively, aggregates of the Aβ peptide could accumulate negative charges on one of its surfaces (Fig. 9B, red spot). This region would interact with multiple positive residues located between the hydrophobic domain and the GXXXG region of the VDAC1 N-terminal domain (Fig. 9B, blue spot). Thus, three contact points could be established between VDAC1 and Aβ. The N-terminal region of VDAC1 was proposed to be flexible, moving in and out of the pore (20). This movement controls VDAC1 conductance and its interaction with anti-apoptotic proteins (17–19, 29, 30). Thus, it may also accessible for interaction with Aβ.

We have clearly demonstrated the ability of the VDAC1 N-terminal peptide to prevent Aβ cell penetration and apo-
ptosis induction. Preincubated Aβ, resembling oligomeric/fibrillar structures but not non-incubated Aβ, induced cell toxicity, which was mediated via both extracellular and intracellular Aβ (3). As the VDAC1 N-Ter peptide used here contains no cell-penetrating sequence, in contrast to our previous studies (55), we suggest that the protection offered by VDAC1 N-Ter peptide against Aβ cell toxicity results from its interaction with extracellular Aβ, preventing its action at the cell surface and penetration into the cell (Fig. 9C). In this respect it was shown recently that Aβ interacts with several receptors, such as PirB, PrP, NMDAR, and EfhB2, and regulates synaptic plasticity by preventing or promoting downstream signaling (56).

**VDAC1 Mediates Aβ Cytotoxicity**

**DECEMBER 25, 2015 • VOLUME 290 • NUMBER 52**

**JOURNAL OF BIOLOGICAL CHEMISTRY**

**30679**

**FIGURE 8. Aβ induces hexokinase detachment, VDAC1 oligomerization, and cytochrome c release.** A, SH-SYSY cells were transfected to express HK-I-GFP (green). Twenty-four hours later the cells were incubated without (Aa) or with Aβ (5 μM (Ab) or 10 μM (Ac)) for 16 h. The cells were then fixed and stained with DAPI (blue). Mitochondrial-bound HK-I-GFP (Aa, arrows) and detached, cytosolic HK-I-GFP (A, b and c, arrowheads), are indicated. Red arrows indicate fragmented nuclei presenting Aβ-induced apoptosis. Scale bar = 20 μm. B and C, Aβ-induced VDAC1 oligomerization, SH-SYSY (B) and HeLa (C) cells were incubated without or with Aβ (5 μM or 10 μM). Forty-eight hours later the cells were analyzed for VDAC1 oligomerization as revealed by EGS-based cross-linking and immunoblotting using anti-VDAC1 antibodies. Quantitative analysis of the level of VDAC1 dimers relative to actin is presented in relative unit. D and E, SH-SYSY cells were incubated with or without Aβ (5 or 10 μM) for 48 h and analyzed for cytochrome c release using anti-cytochrome c-specific antibodies. SH-SYSY cells were treated with digitonin (0.0015%), and release of Cyto c from mitochondria (Mito) to the cytosolic fraction (Cyto) was analyzed by immunoblotting (D) as described under “Experimental Procedures.” Anti-ATP synthase 5a (ATPsyn5a) antibodies were used to demonstrate the absence of mitochondria in the cytosolic fraction. Cytochrome c release was also analyzed by immunocytochemistry using anti-cytochrome c-specific antibodies. Cells were also stained with DAPI (blue) and visualized by confocal microscopy (E). Cytochrome c staining is significantly reduced in the Aβ-treated cells (Eb and Ec) as compared with the control cells (Ea). Scale bars = 10 μm. RU, relative units.
with the maintenance of mitochondrial respiration and glycolysis equilibrium at the heart of energetic and metabolic homeostasis of the cell.

The interaction of HK with VDAC1 offers the cell several advantages that are lost upon HK detachment. HK bound to VDAC1 offers the enzyme direct access to mitochondrial sources of ATP and greater affinity for Mg²⁺-ATP (16). This is lost upon HK detachment from VDAC1 as induced by Aβ.

Moreover, VDAC1-bound HK is less sensitive to inhibition by its product, glucose 6-phosphate (59). Thus, when detached from VDAC1, HK activity is inhibited at low levels of glucose 6-phosphate. In addition, VDAC1-bound HK acts as an anti-apoptotic protein (29, 30, 59–61). Indeed, disruption of HK binding to VDAC1 by Aβ leads to cell death (Fig. 7A). Finally, mitochondria-associated HK was shown to reduce mitochondrial reactive oxygen species (ROS) generation (63).
Glucose hypometabolism not only limits the metabolic capabilities of the cell, it also leads to oxidative stress, known to play a major role in Alzheimer disease (64). In this respect VDAC1 was shown to mediate transport of ROS, with HK binding to VDAC1, reducing ROS transport (65). The formation of mitochondrial ROS was increased by Aβ-amyloid (66). Thus, detachment of HK from VDAC1 by Aβ can lead to increased ROS efflux from its major site of production, the mitochondria, to the cytosol. Several findings support the participation of VDAC1 in ROS transport from the mitochondrial inter-membrane space to the cytosol. HK-I and HK-II bound to VDAC1 decreased ROS release from mitochondria when overexpressed in HEK cells (67), thereby reducing the intracellular levels of ROS (63, 68). Also, expression of HK-I or HK-II was found to protect against oxidant-induced cell death (68, 69). Thus, detachment of HK from VDAC1 by Aβ could lead to increased ROS generation and release to the cytoplasm, subsequently activating cell death. An association between β-amyloid toxicity, mitochondrial dysfunction, oxidative stress, and neuronal damage and death in Alzheimer disease is well established (70). Thus, by preventing Aβ cell penetration with siRNA or VDAC1-N-terminal peptide, Aβ-induced HK detachment would be prevented as well as subsequent effects on cell energy impairment and apoptosis induction (Fig. 9D).

**VDAC1-mediated Aβ Neurotoxicity: a Proposed Mechanism**—A debate exists as to whether fibrillar aggregates (amyloid) or soluble oligomers of Aβ are the toxic form (71). Considering that Aβ induces cell death via mitochondria dysfunction and accepting that soluble oligomeric Aβ is the cell-penetrating and active form, a major question asks how Aβ penetrates into the cell. We showed that VDAC1 is required for Aβ penetration, as in cells depleted of VDAC1 using siRNA, Aβ cell entry is highly reduced, raising the possibility that Aβ enters the cell via pVDAC1. The increase in bilayer-reconstituted VDAC1 conductance by Aβ could indicate the formation of a large channel composed of VDAC1 and Aβ that mediates Aβ import (Fig. 9C). Indeed, pVDAC1 was proposed previously as a candidate for mediating Aβ cell penetration (50). Accordingly, VDAC1-C4AX targeted to plasma membrane induced cell death and added to Aβ toxicity (Fig. 8).

Recent studies revealed that VDAC1 levels are elevated in the affected regions of AD brains and that these elevated VDAC1 levels are linked to the presence of Aβ and phosphorylated Tau, leading to mitochondrial dysfunction in AD neurons (22). It is possible that the elevated VDAC1 levels include a population that is located in the plasma membrane. A model assuming interaction of Aβ peptides with pVDAC1 encourages pVDAC1 oligomerization, leading to peptide entry into the cell, where it induces VDAC1 oligomerization, allowing for cytochrome c release and subsequent apoptosis, is presented in Fig. 9C.

VDAC1 is a crucial factor for mitochondria-mediated apoptosis, with several studies demonstrating that exogenous over-expression of VDAC1 results in cell death (40–45). Moreover, several cancer treatments and apoptosis inducers were found to increase VDAC1 expression levels (16, 44, 72). As VDAC1 over-expression leads to apoptotic cell death, the observed high levels of VDAC1 in the affected regions of AD brains suggest that apoptosis is activated in these regions (21, 22). Our results revealed that Aβ-treated cells show characteristic features of apoptosis, including release of Cyto c, membrane blebbing, and nuclear condensation and fragmentation. In addition, Aβ induced VDAC1 oligomerization. We have shown that VDAC1 oligomerization is coupled to apoptosis induction, with VDAC1 overexpression encouraging its oligomerization, leading to apoptosis in the absence of any apoptosis inducer (16, 44, 48). Mitochondrial-bound HK-I is expressed at particularly high levels in the brain, a tissue well known for its essentially total reliance on the glycolytic metabolism of glucose to sustain its high rate of energy metabolism (73). HK-I also functions as an anti-apoptotic protein (15, 19, 29, 30). We thus propose that displacement of HK-I from VDAC1 encourages VDAC1 oligomerization due to the high concentration of free VDAC1 molecules, thereby leading to apoptosis (Fig. 9C). HK displacement from VDAC1 by Aβ would lead to both apoptosis induction and perturbation of cell energy metabolism.

Having deciphered one of the possible mechanisms of the Aβ-induced neuronal cytotoxicity, we suggest that hampering the VDAC1-Aβ interaction would ameliorate the neurodegeneration seen in AD. This could be achieved by either down-regulating overexpressed VDAC1 RNA levels via siRNA or by inhibiting the VDAC1-Aβ interaction using the VDAC1 N-Ter peptide (Fig. 9D). These approaches may provide new and exciting therapies for AD specifically and possibly for other neurodegenerative diseases.

To conclude, the findings presented here suggest that Aβ-mediated toxicity involves mitochondrial impairment mediated via interaction of Aβ with the VDAC1 N-terminal domain and Aβ penetration into the cell via pVDAC1. Currently, no effective treatment is available for AD, although the Food and Drug Administration has approved several drugs (donepezil, rivastigmine, galantamine, tacrine, and memantine) targeting this condition. These, however, only provide relief from symptoms, and none alters the underlying course of

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**FIGURE 9.** Model describing how Aβ leads to mitochondria-mediated cell death. A, molecular dynamics simulation of the interaction between monomeric Aβ peptide (Gly-29–Ala-42) and the N-terminal domain of VDAC1 (Met-1–Gly-26) identifies two sites of interactions, one of which is the GXGG motif. B, Aβ peptide (Leu-17–Ala-42 and VDAC1 possibly interact through electrostatic (negative charges in Aβ and positive charges in N-terminal domain of VDAC1 are presented by red and blue colors, respectively), hydrophobic, and/or via GXGG motif interactions. The GXGG motif in VDAC1 and the Aβ oligomers are colored green. C, proposed model for Aβ cell penetration and apoptosis induction. Monomeric Aβ can form protofibrils and soluble aggregates. Based on our results, we suggest that VDAC1 plays a key role in Aβ toxicity on several levels. In the plasma membrane, pVDAC1 interacts with Aβ oligomers via its N-terminal domain, leading to VDAC1 oligomerization and the formation of large pores composed of Aβ–VDAC1 heteromers. These large pores allow Aβ to enter the cell and interact with mitochondrial VDAC1, leading to detachment of VDAC1-bound HK-I and VDAC1 oligomerization, concomitant with the formation of heteromeric Aβ–VDAC1 large pores that allow release of Cyto c, and the subsequent induction of apoptosis. Non-membrane-penetrating VDAC1 N-Ter peptides inhibit Aβ cell entry, probably by interacting with Aβ and thus sequestering and preventing Aβ interacting with pVDAC1 and entering the cell to elicit a toxic effect. D, proposed model for VDAC1-based treatment in Alzheimer disease. VDAC1 is overexpressed in Alzheimer disease, leading to VDAC1 oligomerization and subsequent Cyto c release and apoptosis. This VDAC1-dependent apoptosis can be inhibited by preventing VDAC1 expression by VDAC1-siRNA or inhibiting VDAC1 oligomerization stimulated by Aβ by VDAC1-N-Ter peptide.
this terminal disease (62). The direct interaction of Aβ with VDAC1, the requirement of VDAC1 for Aβ-mediated cytotoxicity, and the ability of a VDAC1-derived peptide and reduction in VDAC1 levels to prevent Aβ cytotoxicity point to VDAC1 as a new target for the development of anti-Aβ toxicity drugs for treating AD.

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