C-terminal fragment of N-cadherin accelerates synapse destabilization by amyloid-β

Aksana Andreyeva,1 Katja Nieweg,1 Katharina Horstmann,1 Simon Klapper,1 Andreas Müller-Schiffmann,2 Carsten Korth2 and Kurt Gottmann1

1 Institute of Neuro- and Sensory Physiology, Medical Faculty, Heinrich-Heine-University Düsseldorf, 40225 Düsseldorf, Germany
2 Institute of Neuropathology, Medical Faculty, Heinrich-Heine-University Düsseldorf, 40225 Düsseldorf, Germany

Correspondence to: Dr Kurt Gottmann,
Institut für Neuro- und Sinnesphysiologie,
Gebäude 22.03, Heinrich-Heine-Universität Düsseldorf,
40225 Düsseldorf, Germany
E-mail: kurt.gottmann@uni-duesseldorf.de

The aetiology of Alzheimer’s disease is thought to include functional impairment of synapses and synapse loss as crucial pathological events leading to cognitive dysfunction and memory loss. Oligomeric amyloid-β peptides are well known to induce functional damage, destabilization and loss of brain synapses. However, the complex molecular mechanisms of amyloid-β action resulting ultimately in synapse elimination are incompletely understood, thus limiting knowledge of potential therapeutic targets. Under physiological conditions, long-term synapse stability is mediated by trans-synaptically interacting adhesion molecules such as the homophilically binding N-cadherin/catenin complexes. In this study, we addressed whether inhibition of N-cadherin function affects amyloid-β-induced synapse impairment. We found that blocking N-cadherin function, both by specific peptides interfering with homophilic binding and by expression of a dominant-negative, ectodomain-deleted N-cadherin mutant, resulted in a strong acceleration of the effect of amyloid-β on synapse function in cultured cortical neurons. The frequency of AMPA (alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid) receptor-mediated miniature excitatory postsynaptic currents was reduced upon amyloid-β application much earlier than observed in controls. We further hypothesized that ectodomain-shed, transmembrane C-terminal fragments that are generated during N-cadherin proteolytic processing might similarly enhance amyloid-β-induced synapse damage. Indeed, expression of human N-cadherin C-terminal fragment 1 strongly accelerated amyloid-β-triggered synapse impairment. Ectodomain-shed N-cadherin C-terminal fragment 1 is further proteolytically cleaved by γ-secretase. Therefore, both pharmacological inhibition of γ-secretase and expression of the dominant-negative presenilin 1 mutant L166P were used to increase the presence of endogeneous N-cadherin C-terminal fragment 1. Under these conditions, we again found a strong acceleration of amyloid-β-induced synapse impairment, which could be compensated by over-expression of full-length N-cadherin. Intriguingly, western blot analysis of post-mortem brains from patients with Alzheimer’s disease revealed an enhanced presence of N-cadherin C-terminal fragment 1. Thus, an inhibition of N-cadherin function by proteolytically generated N-cadherin C-terminal fragment 1 might play an important role in Alzheimer’s disease progression by accelerating amyloid-β-triggered synapse damage.

Keywords: Alzheimer’s disease; glutamatergic synapses; N-cadherin; amyloid-β peptide; γ-secretase

Abbreviations: AMPA = alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid; EGFP = enhanced green fluorescent protein; mEPSC = miniature excitatory postsynaptic current; NcadCTF = N-cadherin C-terminal fragment
Introduction

In Alzheimer’s disease, cognitive impairment correlates only weakly with the characteristic senile plaques and neurofibrillary tangles in post-mortem brains but remarkably well with the loss of synaptic structures (Terry et al., 1991). In addition, an impairment of long-term potentiation, a form of synaptic plasticity thought to underlie learning and memory, has been described (Walsh et al., 2002; Walsh and Selkoe, 2007), leading to the hypothesis that synaptic failure is a major early event in the aetiology of Alzheimer’s disease (Selkoe, 2002; Haass and Selkoe, 2007). However, the exact molecular mechanisms involved in synaptic dysfunction and synapse loss are still incompletely understood.

Oligomeric amyloid-β peptides are well known to induce synaptic failure and trigger destabilization of synapses (Walsh et al., 2002; Haass and Selkoe, 2007; Walsh and Selkoe, 2007; Knobloch and Mansuy, 2008). Amyloid-β peptides are major components of senile plaques and are created by proteolytic processing of the transmembrane amyloid-β precursor protein (APP) involving β- and γ-secretase (Hardy and Selkoe, 2002; Haass and Selkoe, 2007; De Strooper, 2010). The γ-secretase complex is made up of several protein subunits with the presenilins being the major catalytic component (Haass and Selkoe, 2007; De Strooper, 2010).

Injection of amyloid-β oligomers into the rat brain has been demonstrated to potently inhibit long-term potentiation in the hippocampus (Walsh et al., 2002; Shankar et al., 2008). Moreover, an enhancement of long-term depression by amyloid-β oligomers has been observed (Shankar et al., 2008; Li et al., 2009a). These findings indicate that amyloid-β oligomers might trigger signalling cascades that are also involved in the induction of long-term depression (Palop and Mucke, 2010). Furthermore, a functional silencing of synapses is indicated by the repeatedly observed amyloid-β-induced loss of miniature excitatory postsynaptic currents (mEPSCs) (Chang et al., 2006; Shankar et al., 2007; Nimmrich et al., 2008; Parodi et al., 2010). In addition to postsynaptic downregulation of AMPA (alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid) receptors (Hsieh et al., 2006; Ting et al., 2007), presynaptic impairments in vesicle cycling have been suggested as underlying mechanisms (Kelly and Ferreira, 2007; Ting et al., 2007; Priller et al., 2009). In addition to alterations in synapse function, amyloid-β oligomers induce synapse destabilization resulting in the ultimate loss of synaptic structures (Walsh and Selkoe, 2007; Koffie et al., 2009). Amyloid-β triggered loss of both presynaptic vesicle clusters and postsynaptic spines has been found (Buttini et al., 2005; Hsieh et al., 2006; Shankar et al., 2007; Evans et al., 2008).

Although the mechanisms of long-term stability of synapses are still not completely understood, it appears conceivable that the state of the long-term maintenance system within a synapse determines its susceptibility to amyloid-β-induced damage. Synapses are thought to be physiologically stabilized through transynaptically interacting adhesion molecules aligning and linking the pre- and postsynaptic compartments (Gottmann, 2008; Südhof, 2008; Shen and Scheiffele, 2010). Neuronal (N)-cadherin is a prototypic synaptic cell adhesion molecule at mature glutamatergic synapses (Benson and Tanaka, 1998; Elste and Benson, 2006; Takeichi, 2007). Postsynaptic N-cadherin interacts homophilically with presynaptic N-cadherin across the synaptic cleft via the extracellular cadherin domains (Takeichi, 2007; Tai et al., 2008). At its cytoplasmic C-terminal domain, N-cadherin binds several types of catenins, e.g. p120 catenin and β-catenin, thus enabling further signal transduction to the actin cytoskeleton (Kwiatkowski et al., 2007; Arrikath and Reichardt, 2008).

Functionally, N-cadherin is involved in the control of presynaptic vesicle accumulation and in vesicle cluster maintenance (Togashi et al., 2002; Bamji et al., 2003; Bozdagi et al., 2004; Stan et al., 2010). In addition, N-cadherin plays an important role in regulating the morphological maturation of postsynaptic spines (Togashi et al., 2002; Arrikath and Reichardt, 2008; Tai et al., 2008). At mature glutamatergic synapses, N-cadherin is modulating short-term plasticity of vesicle release (Togashi et al., 2002; Bozdagi et al., 2004; Jängling et al., 2006) and is required for the induction of long-term potentiation (Tang et al., 1998; Bozdagi et al., 2010).

Furthermore, the transmembrane protein N-cadherin is well known to be proteolytically processed in a similar manner as established for APP (Marambaud et al., 2003; Reiss et al., 2005; Uemura et al., 2006). In the first step, the ectodomain of N-cadherin is shed by the membrane-associated metalloproteinase ADAM10 (Reiss et al., 2005; Uemura et al., 2006; Malinverno et al., 2010). The resulting N-cadherin C-terminal fragment 1 (NcadCTF1) is then further cleaved by γ-secretase/presenilin 1 resulting in the release of NcadCTF2 from the membrane into the cytoplasm (Georgakopoulos et al., 1999; Marambaud et al., 2003; Uemura et al., 2006).

In this study, we wanted to address whether amyloid-β oligomer-induced synapse impairment is influenced by the state of synaptic adhesion mediated by N-cadherin/catenin complexes. Inhibition of N-cadherin function resulted in a strong acceleration of amyloid-β-triggered synapse impairment. The same effect on synapse impairment was observed upon expression of human NcadCTF1 and upon inhibition of γ-secretase, which led to an increase in endogeneous NcadCTF1. Most interestingly, NcadCTF1 presence was found to be increased in the post-mortem brains of patients with Alzheimer’s disease, suggesting that NcadCTF1 might enhance amyloid-β-triggered synapse damage in Alzheimer’s disease by inhibiting N-cadherin function.

Materials and methods

Cell culture and transfection

Cultures of cortical neurons were prepared from C57BL/6J mouse foetuses at embryonic Day 18 as described previously (Mohrmann et al., 2003; Stan et al., 2010), except that no glial cells and no cytotoxic arabinoside (ARAC) were added. Neurons were grown in Neurobasal® A medium (Invitrogen) supplemented with 2% N2-1, 100 U/ml penicillin, 100 μg/ml streptomycin and 1 mM GlutaMAX™ (Invitrogen) on glass coverslips (for immunocytochemistry and electrophysiology) or in tissue culture dishes (for biochemistry), both coated with poly-L-ornithine (1 mg/ml). Neurons were transfected at 7 days in vitro using Lipofectamine® 2000 (Invitrogen) according to the manufacturer’s instructions. Cotransfection with an enhanced green
fluorescent protein (EGFP) expression vector (pEGFP-N1) was done to visualize transfected neurons by EGFP fluorescence. At 8–10 days in vitro, amyloid-β containing 7PA2 conditioned medium, immunodepleted 7PA2 conditioned medium or control medium was added to the cultures by 1:1 dilution into standard medium. Electrophysiologically analysis was done at 10–13 days in vitro.

For western blot detection of NcadCTF1 accumulation, cultured cortical neurons were treated at 8 days in vitro by the γ-secretase inhibitor L-685,458 (5 μM) and at 10 days in vitro with amyloid-β containing 7PA2 conditioned medium or culture medium (control). At 12 days in vitro, cells were washed twice with cold PBS, scraped into 10 mM MOPS [3-(N-Morpholino)propanesulfonic acid], pH 7.0/10 mM KCl/10 mM CaCl₂/10 mM EGTA (ethylene glycol tetraacetic acid) and homogenized. Protein concentration was determined by bicinchoninic acid (BCA) Protein Assay Kit (Thermo Scientific Pierce). The homogenates were boiled in Laemmli buffer and analysed by SDS-PAGE and western blot.

Human neural stem cells were derived from induced pluripotent stem cells (Zaehres et al., 2010). Therefore, neuroepithelial clusters generated according to Li et al. (2009b) were maintained in suspension culture for 2 weeks in N2827 medium [Dulbecco’s modified Eagle medium/F12, penicillin/streptomycin, 2 mM GlutaMAX™, B27 without vitamin A, N2 supplement, 50 μg/ml bovine serum albumin (all Invitrogen), 20 μg/ml insulin (Sigma Aldrich)] with human recombinant epidermal growth factor and basic fibroblast growth factor (10 ng/ml, Peprotech). After dissociation with Accutase® (Sigma Aldrich), neural stem cells were kept as adherent culture on polyornithine/laminin-coated tissue culture plastics in the same conditions and passed every 5 days with Accutase®. For western blot detection of NcadCTF1 accumulation, neural stem cells were treated with L-685,458 (5 μM) for 6 h and then further processed as described above for cultured cortical neurons.

In vitro differentiation, immunoisolation and cultivation of mouse embryonic stem cell-derived N-cadherin knockout neurons were performed as described (Jüngling et al., 2003; Stan et al., 2010), except that neurons were cultured on coverslips coated with poly-L-ornithine (1 mg/ml).

**Constructs, peptides and inhibitors**

The pEGFP-N1 vector was commercially obtained (Clontech). pcS2-NcadΔE-myc construct was a kind gift from Dr C. Holt (Cambridge, UK). Full-length N-cadherin expression vector (pMS149.1) was kindly provided by Dr S. Weggen (Düsseldorf, Germany).

For pDNA3.1-NcadCTF1 and pDNA3.1-FLAG-NcadCTF1, N-cadherin sequence was amplified from a full open reading frame expression N-cadherin clone OCAao5051E01108-pdEYFP-C1amp (iGenome). For PCR amplification of the signal peptide sequence (amino acids -159 to -133, EMBL accession no. X94153), a forward primer 5’-CATATAGCCGCGGCCTCCTGATCGGCGATCCGGAG C-3’ and a reverse primer 5’-CATATACGGAGCTCCCTACAGA CGC-3’ were used. For PCR amplification of the sequence encoding CTF1 (amino acids 557 to 747, EMBL accession no. X54315), a forward primer 5’-CATATACGGTGGGCTCGGGCTCGGACCGGT GC-3’ and a reverse primer 5’-CATATAGCCCCCTCCATGCTCACC CCCACGATCGTGC-3’ were used. For N-terminal FLAG-tagged NcadCTF1, a forward primer 5’-CATATACGGGATCCATAGAAGAT GACCGACGATAAAGTATCCCTCCAAATACAAATTTCC-3’ was used (amino acids 527 to 747). The PCR products were sequentially cloned into NotI-Xhol and Xhol-Apal sites of pcDNA3.1(+) vector (Invitrogen). Precise cloning of the reading frame of all constructs was verified by sequencing.

INPISOQ (N-cadherin function blocking; Williams et al., 2000) and LVRIRS (control; Williams et al., 2000) synthetic peptides (PolyPeptide Laboratories) were dissolved at 1 mg/ml in 0.1% acetic acid and used at a final concentration of 100 μg/ml.

L-685,458 (γ-secretase inhibitor X) (Calbiochem) was dissolved in dimethyl sulphoxide (DMSO) according to the manufacturer’s instruction at a concentration of 1 mM and diluted into culture medium at a final concentration of 5 μM. DMSO controls contained 0.5% DMSO.

**Amyloid-β containing 7PA2 conditioned medium and synthetic amyloid-β-42**

Conditioned medium containing naturally secreted amyloid-β peptides was obtained from a Chinese hamster ovary cell line (7PA2 cells) that stably expresses human APP751 carrying the familial APP V717F mutation (Podlisny et al., 1995; Walsh et al., 2002). The 7PA2 cells were cultured in Dulbecco’s modified Eagle medium supplemented with 10% foetal bovine serum, 1 mM sodium pyruvate, 100 U/ml penicillin and 100 μg/ml streptomycin (Invitrogen). To obtain conditioned medium, confluent cultures were incubated with Neurobasal® A medium (Invitrogen) supplemented with 2% NS-21, 100 U/ml penicillin, 100 μg/ml streptomycin and 1 mM GlutaMAX™ (Invitrogen) for 72 h. Then, cells were removed by centrifugation and the conditioned medium was stored at −80°C. 7PA2 conditioned medium contained amyloid-β peptides at a concentration of 25–50 ng/ml as determined by ELISA (amyloid-β-40).

We immunodepleted 1 ml 7PA2 conditioned medium with 15 μg anti-amyloid-β monoclonal antibody (IC16; Müller-Schiffmann et al., 2010) coupled to NHS-Sepharose™ (GE Healthcare) by overnight incubation at 4°C followed by centrifugation.

Synthetic amyloid-β (1-42) (Bachem) was dissolved at a concentration of 10 μM in Neurobasal® A medium (Invitrogen) supplemented with 2% NS-21, 100 U/ml penicillin, 100 μg/ml streptomycin and 1 mM GlutaMAX™ and kept frozen until use. Amyloid-β (1-42) was added to cultures at a final concentration of 1 μM. Amyloid-β oligomers were detectable by western blot after 4 days of incubation with cultured cortical neurons (Supplementary Fig. 2).

**Electrophysiology and data analysis**

Whole-cell patch-clamp recordings were performed in cultured cortical neurons at room temperature using an EPC7 patch-clamp amplifier (HEKA) and pCLAMP software (Molecular Devices) as described previously (Mohrmann et al., 1999; Jüngling et al., 2006). About three to five cells were recorded per coverslip; for each experiment, at least four independent cell preparations were studied. Patch electrodes (7–8 MΩ) were filled with an intracellular solution containing 110 mM KCl, 0.25 mM CaCl₂, 10 mM EGTA (ethylene glycol tetraacetic acid) and 20 mM HEPES [4-(2-hydroxyethyl)-1-piperazinethanesulfonic acid], pH 7.3. The standard extracellular solution contained 130 mM NaCl, 5 mM KCl, 2.5 mM CaCl₂, 1 mM MgCl₂ and 20 mM HEPES, pH 7.3. To pharmacologically isolate AMPA receptor-mediated mEPSCs (AMPA-mEPSCs), tetrodotoxin (1 μM) and gabazine (10 μM) were added to the extracellular solution. AMPA mEPSCs were recorded under voltage-clamp conditions at a holding potential of −60 mV and were completely blocked by addition of 6,7-dinitroquinoxaline-2,3-dione (10 μM). In general, AMPA mEPSC...
frequencies appeared to be relatively low, most likely because the cortical cultures from embryonic Day 18 foetuses contained only few glial cells, which are well known to enhance mEPSC frequency (Slezak and Pfrieger, 2003).

Quantitative analysis of AMPA mEPSC frequencies and amplitudes was performed using Mini Analysis Software (Synaptosoft). For detection of mEPSCs, an amplitude threshold of 5 pA was used. Whole-cell membrane capacitance was determined by integrating capacitative current transients using Clampfit software (Molecular Devices).

**Immunocytochemistry, fluorescence microscopy and analysis of cell death**

Cortical neurons and embryonic stem cell-derived N-cadherin knock-out neurons cultured on glass coverslips were fixed with 4% paraformaldehyde in PBS for 15 min and subsequently permeabilized and blocked with 0.25% Triton X-100/30% goat serum in PBS for 45 min at room temperature. Cells were then incubated with primary antibodies—rabbit polyclonal anti-vesicle-associated membrane protein 2 (VAMP2) antibody (1/500, Synaptic Systems) and chicken polyclonal anti-microtubule-associated protein 2 (MAP2) antibody (1/500, ab92434, AbCam); mouse monoclonal anti-FLAG® antibody (1/800, M2, Sigma); mouse monoclonal anti-N-cadherin antibody (1/200, C32, BD Transduction Laboratories)—diluted in 3% goat serum/PBS for 1 h at room temperature. Cells were washed with 0.1% Tween-20 in PBS, followed by 50 min incubation at room temperature with secondary antibodies—anti-rabbit Cy3 (1/10000, Chemicon); anti-chicken AMCA (7-amino-4-methylcoumarin-3-acetic acid, 1/50, Jackson ImmunoResearch Laboratories); anti-mouse Alexa Fluor® 555 (1/1500, Invitrogen)—diluted in 3% goat serum/PBS. Coverslips were washed with PBS and mounted with FluorSave™ Reagent (Calbiochem).

Fluorescence microscopy of immunostained neurons and transfected EGFP-expressing neurons was done by obtaining fluorescence images (z-stacks of images, if required) using a computer-controlled, motorized Zeiss Axiovert 200M inverted fluorescence microscope as described (Stan et al., 2010). Image acquisition was performed with a 12-bit monochrome CoolSNAP ES2 CCD camera (Photometrics) by using MetaVue software (Visitron Systems). For image analysis, 3D deconvolution of z-stacks was done by using AutoDeblur Software (Visitron Systems) as described (Stan et al., 2010). Maximum intensity projection images of z-stacks were thresholded by using MetaMorph software (Visitron Systems) to obtain fluorescent VAMP2 immunostained puncta. Quantitative analysis of VAMP2 immunostained vesicle clusters was carried out by counting thresholded VAMP2 puncta on MAP2 immunostained proximal dendrites.

Cell viability was studied by using the LIVE/DEAD® Viability/Cytotoxicity Kit (Molecular Probes) according to the manufacturer’s instructions. In brief, healthy neurons were stained with membrane-permeable Calcein-AM, which is intracellularly converted to fluorescent calcein. Nuclei of cells without intact cell membrane were stained with ethidium homodimer-1. The fraction of healthy neurons was determined by counting both calcein-stained cell somata and ethidium homodimer-1-stained nuclei of dead cells in fluorescence images.

**Western blot analysis**

Proteins in the homogenates prepared from cortical cultured neurons, human neural stem cells and human brain samples were separated by 12% SDS–PAGE and electroblotted onto 0.2 µm nitrocellulose membrane Protran® (Schleicher and Schuell) for 2 h at 90 V. Blots were incubated with rabbit polyclonal anti-actin antibody (Sigma), mouse monoclonal anti-N-cadherin antibodies C32 (BD Transduction Laboratories) and 389 (Invitrogen) followed by incubation with IRDye®-labelled secondary antibodies.

For detection of amyloid-β peptides in 1 ml 7PA2 conditioned medium, amyloid-β species were immunolocalized with 1 µg IC16 antibody coupled to NHS-sepharose (Müller-Schiffmann et al., 2010) by incubation for 3 h at 4°C. Immunoprecipitates were washed with 50 mM Tris–HCl buffer (pH 7.5) and subjected to western blot. Amyloid-β species were separated using 16.5% Tris–Tricine precast gel (BioRad) and electroblotted onto 0.2 µm nitrocellulose membrane Protran® (Schleicher and Schuell) for 2 h at 90 V. Nitrocellulose membrane was boiled in PBS for 5 min before incubation with 6E10 anti-amyloid-β antibody (Covance) followed by incubation with IRDye®-labelled secondary antibodies.

Secondary antibodies against mouse and rabbit immunoglobulins conjugated with IRDye® 680 or IRDye® 800CW were detected by the ODYSSEY® Infrared Imaging System (LI-COR Biosciences). Molecular weight markers were prestained protein standards and polypeptide standards (Bio-Rad). Blots were quantified using ODYSSEY® Infrared Imaging System software (LI-COR Biosciences).

**Post-mortem human brain samples**

Post-mortem human brain samples (superior frontal gyrus) were provided by the Netherlands Brain Bank (Amsterdam, The Netherlands; http://www.brainbank.nl). Additional samples were obtained via the European BrainNet after informed consent and with consideration of all relevant ethical issues (outlined in http://www.brainnet-europe.org). Tissue from six patients with Alzheimer’s disease (Braak stages V–VI; 80- to 87-year-old females), four patients with Alzheimer’s disease (Braak stages III–VI; 75- to 90-year-old females) and from six non-demented individuals (Braak stages I–II; five 80- to 87-year-old females and one 85-year-old female) was used. Experimentation with post-mortem human brain tissue was approved by the Ethics Committee of the Medical Faculty of the University of Düsseldorf.

Brain homogenates (10% w/v) were prepared from frozen brain pieces in ice-cold VRL buffer (50 mM HEPES, pH 7.5, 250 mM sucrose, 5 mM MgCl2, 100 mM KCH3CO2, complete protease inhibitor cocktail (Roche Diagnostics)) with a potter homogenizer. Homogenates were immediately frozen on dry ice and stored at −80°C. Aliquots (50 µl) of the homogenates were sonicated for 30 s with an amplitude of 40% in a Misonix S-4000 sonicator (QSonica). Protein concentrations were measured in quadruple using the BCA-kit (Bio-Rad). Each homogenate (30 µg) was boiled in Laemmli buffer and separated on a 4–12% Bis-Tris gel (Invitrogen) and proteins were then transferred on a 0.2 µm NC-membrane for 1.5 h at 400 mA for western blot analysis. Western blot signals of NCadCTF1 and b-actin were quantified by the ImageJ software (http://rsb.info.nih.gov/iij/), and densitometric units of NCadCTF1 were calculated after normalization with the corresponding β-actin signals.

**Statistical analysis**

Results are given as means ± SEM. Statistical significance was determined using Student’s t-test. Significance levels were Bonferroni-corrected where required (Supplementary Table 1).
Results

Inhibition of N-cadherin adhesion accelerates amyloid-β-induced impairment of glutamatergic synapse function

To study the role of N-cadherin-mediated trans-synaptic adhesion in amyloid-β-induced synapse impairment, we first characterized the effects of amyloid-β peptides on glutamatergic synapses in cultured mouse cortical neurons. We incubated the cultured neurons with conditioned medium from cultures of the 7PA2 Chinese hamster ovary cell line (1:1 diluted with standard culture medium), which is well known to contain toxic amyloid-β oligomers (Podlisny et al., 1995; Walsh et al., 2002; Fig. 1D). Addition of amyloid-β peptides containing 7PA2 conditioned medium was done at 8 days in vitro and synapses were studied electrophysiologically by recording AMPA mEPSCs over the following 3 days. At 2 days after amyloid-β addition, no significant differences in the mean frequency or amplitude of AMPA mEPSCs were found. In contrast, at 3 days after amyloid-β addition, the mean frequency of AMPA mEPSCs was strongly reduced (P < 0.01; Fig. 1A and B). The mean cell membrane capacitance was not significantly altered (Supplementary Fig. 1A and B), indicating that the reduction in AMPA mEPSC frequency was not caused by a loss of dendrites. In addition, at 3 days after amyloid-β addition, the mean amplitude of AMPA mEPSCs was slightly reduced (Fig. 1A and B). Immunodepletion of the 7PA2 conditioned medium with amyloid-β-specific antibodies (IC16 antibody; Fig. 1D) blocked both effects on AMPA mEPSCs, indicating that amyloid-β peptides were the active compounds in the 7PA2 conditioned medium. Although the immunocytochemical staining of synaptic vesicle clusters (VAMP2) on dendrites revealed a significant (P < 0.001) reduction in the dendritic density of VAMP2 puncta at 3 days after amyloid-β addition (Fig. 1C), this slight synapse loss was too small to fully account for the dramatic reduction in AMPA mEPSC frequency. We also addressed neuron survival by staining healthy neurons with the intracellularly trapped fluorescent dye calcein (live/dead assay). Amyloid-β-induced neuron death became prominent only 4 days after amyloid-β addition, although a slight neuron loss was also present at 3 days after amyloid-β addition.

Figure 1 Oligomeric amyloid-β containing conditioned medium of the 7PA2 Chinese hamster ovary cell line impairs glutamatergic synapse function after 3 days of incubation in cultured cortical neurons. Note the absence of amyloid-β effects at 2 days of incubation. (A) Example traces of AMPA receptor-mediated mEPSCs (AMPA mEPSCs) 3 days after addition (at 8 days in vitro) of non-conditioned culture medium (vehicle), amyloid-β containing culture medium conditioned by the 7PA2 Chinese hamster ovary cell line (7PA2) and amyloid-β-depleted culture medium conditioned by the 7PA2 Chinese hamster ovary cell line (depleted 7PA2) to cultured cortical neurons. (B) Quantification of AMPA mEPSC frequencies (top) and AMPA mEPSC amplitudes (bottom) after 2 days and after 3 days of incubation with amyloid-β. (C) Quantification of the dendritic density of VAMP2 immunostained vesicle clusters at 2 and 3 days of incubation with amyloid-β. (D) Western blot of amyloid-β peptides pulled down with bead-attached IC16 antibodies from medium conditioned by the 7PA2 Chinese hamster ovary cell line (7PA2) and from immunodepleted conditioned medium (depleted 7PA2). Blot was probed with 4G8 antibody. Bars represent means ± SEM. n (cells) is indicated on bars. Cells/cultures used: (B) 27/12, 24/10; 32/13, 27/12, 24/13; (C) 24/6; 22/6; 25/6, 23/6, 23/6. **P < 0.01 Student’s t-test (with Bonferroni correction, if applicable).
Thus, our results demonstrate that amyloid-\(\beta\) peptides containing 7PA2 conditioned medium induced an initially functional defect at glutamatergic synapses, which first became detectable 3 days after amyloid-\(\beta\) addition in cultured cortical neurons.

To inhibit N-cadherin-mediated trans-synaptic adhesion, we used function blocking INP peptides, which mimic a short sequence in the EC1 domain of N-cadherin and thus impair the homophilic trans-synaptic interaction of N-cadherin molecules (Tang et al., 1998; Williams et al., 2000). Blocking INP peptides and control peptides were added at 8 days \textit{in vitro} at a concentration of 100 \(\mu\)g/ml. In control experiments, blocking INP peptides alone did not significantly affect AMPA mEPSC frequency even after 3 days of incubation (control: 1.16 \(\pm\) 0.27 Hz, \(n=19\); INP: 0.68 \(\pm\) 0.24 Hz, \(n=19\)). In line with the observed tendency to a reduction of AMPA mEPSC frequency by INP peptides, N-cadherin knockout neurons also exhibited a rather low AMPA mEPSC frequency (0.46 \(\pm\) 0.12 Hz, \(n=31\)). To study whether inhibition of N-cadherin alters amyloid-\(\beta\) effects on synapses, amyloid-\(\beta\) peptides containing 7PA2 conditioned medium was added together with INP peptides at 8 days \textit{in vitro} and AMPA mEPSCs were recorded after 2 days. Intriguingly, at 2 days after the addition of amyloid-\(\beta\), the mean frequency of AMPA mEPSCs was already strongly (\(P=0.013\)) reduced in the presence of blocking INP peptides, whereas amyloid-\(\beta\) addition did not yet exhibit any effect in the presence of control peptides (Fig. 2A–C). Thus, a strong acceleration of the effect of amyloid-\(\beta\) on AMPA mEPSC frequency was found upon inhibition of N-cadherin function. AMPA mEPSC amplitudes were not affected by amyloid-\(\beta\). The presence of blocking INP peptides did not alter cell membrane capacitance (Fig. 2D), indicating that cell viability was not affected. In addition, the immunocytochemical staining of synaptic vesicle clusters (VAMP2) on dendrites revealed a significant (\(P<0.05\)) reduction in the dendritic density of VAMP2 puncta as soon as 2 days after amyloid-\(\beta\) addition in the presence of blocking INP peptides (Fig. 2E). In summary, these results strongly suggest that inhibition of N-cadherin-mediated trans-synaptic adhesion leads to an acceleration of the amyloid-\(\beta\)-induced impairment of synapses.

**Inhibition of N-cadherin function by expression of an N-cadherin C-terminal fragment accelerates amyloid-\(\beta\)-induced impairment of glutamatergic synapse function**

To further investigate the role of N-cadherin in amyloid-\(\beta\)-induced synapse impairment, we inhibited the function of classical cadherins.

---

**Figure 2** Inhibition of N-cadherin function by blocking INP peptides accelerates amyloid-\(\beta\)-induced synapse impairment. (A and B) Example recordings of AMPA mEPSCs 2 days after addition (at 8 days \textit{in vitro}) of non-conditioned culture medium (vehicle) and after addition of amyloid-\(\beta\) containing 7PA2 conditioned medium (7PA2) in cortical neurons treated with control peptides (A) and in cortical neurons treated with blocking INP peptides (100 \(\mu\)g/ml, addition at 8 days \textit{in vitro}) (B). (C and D) Quantification of AMPA mEPSC frequencies (C, \textit{left}), AMPA mEPSC amplitudes (C, \textit{right}) and cell membrane capacitance (D) after 2 days of incubation with vehicle or amyloid-\(\beta\) containing 7PA2 conditioned medium. Note the accelerated effect of amyloid-\(\beta\) on AMPA mEPSC frequency upon inhibition of N-cadherin function by INP peptides. (E) Quantification of the dendritic density of VAMP2 immunostained vesicle clusters (normalized to mean of control) at 2 days of incubation with vehicle (control) or amyloid-\(\beta\) containing 7PA2 conditioned medium. Bars represent means \(\pm\) SEM. \(n\) (cells) is indicated on bars. Cells/cultures used: (C) 26/10, 25/11, 27/9, 26/9; (D) 25/10, 24/11, 27/9, 26/9; (E) 26/6, 22/6, 24/6, 26/6. *\(P<0.05\) Student’s t-test.
by expressing a dominant-negative, truncated N-cadherin lacking the extracellular cadherin domains (N-cadherin/C1E). This approach is well established to inhibit the function of endogeneous N-cadherin at synapses (Togashi et al., 2002; Bozdagi et al., 2004; Stan et al., 2010). Individual cells in cultures of mouse cortical neurons were transfected at 7 days in vitro with an expression vector encoding Xenopus N-cadherin/C1E (Riehl et al., 1996) using the Lipofectamine® technique. A cotransfection with EGFP was done to visualize the transfected neurons (Fig. 3A). Two days after transfection, amyloid-β peptides containing 7PA2 conditioned medium were added to the cultures, and AMPA mEPSCs were recorded from transfected neurons 2 days later. Strikingly, at 2 days after amyloid-β addition, the frequency of AMPA mEPSCs was already strongly (P < 0.01) reduced in cells expressing N-cadherin/C1E, whereas no effect of amyloid-β peptides was observed in control EGFP-expressing neurons (Fig. 3B). This result indicates a strong acceleration of amyloid-β-induced synapse impairment upon inhibition of N-cadherin. The effect of amyloid-β on AMPA mEPSC amplitudes was not changed. The morphological appearance of EGFP-expressing cells was not altered upon N-cadherin/C1E expression and amyloid-β addition (Fig. 3A). A slight trend to a reduced cell membrane capacitance was found upon N-cadherin/C1E expression, but amyloid-β addition did not significantly affect cell membrane capacitance (Fig. 3B). Thus, inhibition of N-cadherin function by expression of a dominant-negative, C-terminal fragment of N-cadherin led to a similar acceleration of amyloid-β-induced synapse impairment as found upon inhibition of transynaptic adhesion by blocking peptides.

We wanted to confirm our results further with addition of synthetic amyloid-β42 instead of amyloid-β peptides containing 7PA2 conditioned medium. As indicated by western blot, after 4 days of transfection (using Lipofectamine® at 7 days in vitro) with either EGFP only (control) or with ectodomain-deleted N-cadherin (NcadΔE) + EGFP. Two days of incubation (starting at 9 days in vitro) with vehicle or amyloid-β containing 7PA2 conditioned medium. Scale bars = 100 µm. (B) Upper: Example recordings of AMPA mEPSCs in NcadΔE + EGFP transfected neurons 2 days after addition (at 9 days in vitro) of non-conditioned culture medium (vehicle) and addition of amyloid-β containing 7PA2 conditioned medium (7PA2). Lower: Quantification of AMPA mEPSC frequencies, AMPA mEPSC amplitudes and cell membrane capacitances after 2 days of incubation with vehicle or amyloid-β containing 7PA2 conditioned medium. Note the accelerated effect of amyloid-β on AMPA mEPSC frequency upon inhibition of N-cadherin function by NcadΔE expression. (C) Upper: Example recordings of AMPA mEPSCs in NcadΔE + EGFP transfected neurons 3 days after addition (at 9 days in vitro) of culture medium (vehicle) and addition of synthetic amyloid-β42 (Aβ42) in culture medium (1 µM). Lower: Quantification of AMPA mEPSC frequencies, AMPA mEPSC amplitudes and cell membrane capacitances after 3 days of incubation with vehicle or synthetic amyloid-β42. Bars represent means ± SEM, n (cells) is indicated on bars. Cells/cultures used: (B) 21/13, 23/12; 20/13, 21/12; (C) 23/10, 24/11; 25/10, 25/10. *P < 0.05; **P < 0.01 Student’s t-test.

Figure 3 Inhibition of N-cadherin function by expression of an ectodomain-deleted, dominant-negative N-cadherin mutant accelerates amyloid-β-triggered synapse impairment. (A) Dendritic morphology of individual cultured cortical neurons as revealed by EGFP fluorescence 4 days after low-efficiency
incubation with cultured cortical neurons, the culture medium contained amyloid-β oligomers in addition to monomers (Supplementary Fig. 2E). Addition of synthetic amyloid-β$_{1-42}$ (1 μM) to cultured mouse cortical neurons at 9 days in vitro resulted in a strongly ($P < 0.01$) reduced AMPA mEPSC frequency 4 days after amyloid-β$_{1-42}$ addition, whereas 3 days after amyloid-β$_{1-42}$ addition, no significant effect was observed. Similarly, 4 days after amyloid-β$_{1-42}$ addition, a significantly ($P < 0.05$) reduced AMPA mEPSC amplitude was observed, whereas 3 days after amyloid-β$_{1-42}$ addition, no significant effect was found (Supplementary Fig. 2). No significant changes in the cell membrane capacitance and in the fraction of healthy neurons (live/dead assay) were detectable 4 days after amyloid-β$_{1-42}$ addition, indicating that cell viability was not affected by our synthetic amyloid-β$_{1-42}$ preparation. Next, we studied the effects of inhibition of N-cadherin function on synthetic amyloid-β$_{1-42}$-induced impairment of synaptic function. In N-cadherinΔE-expressing neurons, the frequency of AMPA mEPSCs was already strongly ($P < 0.05$) reduced 3 days after synthetic amyloid-β$_{1-42}$ addition, whereas no amyloid-β effect was observed in EGFP-expressing control neurons (Fig. 3C). The effect of synthetic amyloid-β$_{1-42}$ on AMPA mEPSC amplitudes was not accelerated. Taken together, our experiments with addition of synthetic amyloid-β$_{1-42}$ strongly confirmed that inhibition of N-cadherin function by expression of a dominant-negative, C-terminal fragment of N-cadherin results in an accelerated impairment of synaptic function by amyloid-β peptides.

**Expression of the ectodomain-shed, C-terminal fragment of human N-cadherin accelerates amyloid-β-induced synaptic impairment**

N-cadherin processing involves proteolytic cleavage by both $\alpha$-secretase (ADAM10) and $\gamma$-secretase. Ectodomain shedding by $\alpha$-secretase creates a transmembrane CTF1 that is further degraded by $\gamma$-secretase (Marambaud et al., 2003; Reiss et al., 2005; Uemura et al., 2006). To address whether the physiologically occurring NcadCTF1 has a similar accelerating effect on amyloid-β-induced synaptic impairment as N-cadherinΔE, we expressed human NcadCTF1 in cultured cortical neurons and studied AMPA mEPSCs. Two slightly different NcadCTF1 constructs were made, one with an additional N-terminal FLAG-tag to enable specific detection of expression and another one without FLAG-tag exactly resembling the physiologically occurring NcadCTF1. Cotransfection of individual neurons with these constructs and EGFP was done at 7 days in vitro using the Lipofectamine® technique, and amyloid-β containing 7PA2 conditioned medium was added at 9 days in vitro.

Four days after transfection, expression of FLAG-tagged NcadCTF1 was clearly detectable immunocytochemically in both the cell soma and the dendrites of transfected neurons (Fig. 4A). In FLAG-tagged NcadCTF1-expressing neurons, the frequency of AMPA mEPSCs was already strongly ($P < 0.01$) reduced 2 days after amyloid-β addition, whereas no amyloid-β effect was observed in EGFP-expressing control neurons (Fig. 4B). AMPA mEPSC amplitudes were not affected. No changes in overall cell morphology and cell membrane capacitance were observed (Supplementary Fig. 3A and B), indicating that cell viability was not affected by expression of FLAG-tagged NcadCTF1.

We then expressed the physiologically occurring NcadCTF1 in individual cultured cortical neurons (cotransfection with EGFP at 7 days in vitro) and studied its effect on amyloid-β (added at 9 days in vitro) induced impairment of synaptic function. Expression of NcadCTF1 was confirmed in N-cadherin knockout neurons using antibodies against the C-terminal domain of N-cadherin (Supplementary Fig. 3C). In NcadCTF1-expressing neurons, the frequency of AMPA mEPSCs was again already strongly ($P = 0.025$) reduced 2 days after amyloid-β addition, whereas no amyloid-β effect was observed in EGFP-expressing control neurons (Fig. 4D). AMPA mEPSC amplitudes were not affected. No changes in overall cell morphology (Fig. 4C) and cell membrane capacitance were observed (Supplementary Fig. 3B), indicating that cell viability was not affected by expression of NcadCTF1. In summary, our results with expression of C-terminal fragments of N-cadherin demonstrate that the physiologically occurring, ectodomain-shed NcadCTF1 is able to accelerate the amyloid-β-induced impairment of synaptic function by inhibiting the function of full-length N-cadherin.

**Inhibition of $\gamma$-secretase activity accelerates amyloid-β-induced synapse impairment**

We then wanted to study the effect of an enhanced presence of endogenous NcadCTF1 on the amyloid-β-induced impairment of glutamatergic synapse function. To inhibit the breakdown of endogenous ectodomain-shed NcadCTF1 by $\gamma$-secretase, we added the $\gamma$-secretase inhibitor L-685,458 (5 μM) to cultures of cortical neurons at 8 days in vitro. A strong increase in the amount of ectodomain-shed NcadCTF1 was found by western blot analysis after 4 days of incubation (Fig. 5A). Amyloid-β peptides containing 7PA2 conditioned medium was added at 10 days in vitro and AMPA mEPSCs were recorded 2 days after amyloid-β addition. In L-685,458 incubated cultures, the frequency of AMPA mEPSCs was already strongly ($P = 0.024$) reduced 2 days after amyloid-β addition, whereas no amyloid-β effect was found in DMSO-treated control cultures (Fig. 5B). AMPA mEPSC amplitudes were not altered. No changes in cell survival (live/dead assay) and cell membrane capacitance were observed (Supplementary Fig. 4), indicating that cell viability was not affected by addition of L-685,458. Activation of NMDA (N-methyl-D-aspartate) receptors by coapplication of NMDA (50 μM) with L-685,458 enhanced the amyloid-β-induced reduction of AMPA mEPSC frequency (L-685,458 only: 51% reduction by amyloid-β; L-685,458 + NMDA: 83% reduction by amyloid-β).

We further addressed whether specific strengthening of the N-cadherin adhesion system by over-expression of full-length N-cadherin influences the acceleration of the amyloid-β effect on AMPA mEPSC frequency. We expressed full-length N-cadherin in individual cultured cortical neurons (cotransfection with EGFP at 7 days in vitro) and added the $\gamma$-secretase inhibitor.
L-685,458 (5 μM) at 8 days in vitro. Amyloid-β peptides containing 7PA2 conditioned medium were added at 10 days in vitro and AMPA mEPSCs were recorded 2 days after amyloid-β addition. In N-cadherin over-expressing neurons incubated with L-685,485, no significant change in AMPA mEPSC frequency and amplitudes was induced by amyloid-β peptides after 2 days of incubation (Fig. 5B). These results indicate that the specific over-expression of full-length N-cadherin is sufficient to compensate the acceleration of the effect of amyloid-β peptides on AMPA mEPSC frequency.
We then studied the effect of the expression of a dominant-negative presenilin 1 mutation (L166P), which has been found in familial Alzheimer’s disease and has been described to exhibit a reduced \( \gamma \)/C13-secretase activity thus leading to an increased amount of endogenous \( N \)-cadherin CTF (Bentahir et al., 2006). We expressed PS1-L166P in individual cultured cortical neurons (cotransfection with EGFP at 7 days in vitro) and studied its effect on amyloid-\( \beta \) (added at 9 days in vitro) induced impairment of synaptic function. In PS1-L166P-expressing neurons, the frequency of AMPA mEPSCs was again strongly (\( P = 0.018 \)) reduced 2 days after amyloid-\( \beta \) addition, whereas no amyloid-\( \beta \) effect was observed in EGFP-expressing control neurons (Fig. 5D). AMPA mEPSC amplitudes were not altered. No changes in overall cell morphology (Fig. 5C) and cell membrane capacitance were observed (Supplementary Fig. 4D), indicating that cell viability was not affected by expression of PS1-L166P. Taken together,
our experiments involving inhibition of γ-secretase by pharmacological blockade and by expression of a dominant-negative PS1 mutation indicated that an increased presence of endogeneous NcadCTF1 results in an acceleration of the amyloid-β-induced impairment of glutamatergic synapse function.

Presence of endogeneous NcadCTF1 is enhanced in post-mortem brains of patients with Alzheimer’s disease

The acceleration of the amyloid-β-induced impairment of glutamatergic synaptic function by NcadCTF1 might be of relevance in Alzheimer’s disease if an enhanced presence of NcadCTF1 could be demonstrated in the brains of these patients. We therefore studied the presence of NcadCTF1 in post-mortem brain homogenates from patients with late-onset Alzheimer’s disease by western blotting (Fig. 6A). Western blots were probed with two independent N-cadherin antibodies resulting in the same pattern of bands (Fig. 6D). The band corresponding to human NcadCTF1 was identified by incubating cultured human neural stem cells (derived from induced pluripotent stem cells) with the γ-secretase inhibitor L-685,458 (5 μM) for 6 h. This treatment led to the clear appearance of a western blot band with a molecular weight of ~40 kDa, representing the ectodomain-shed NcadCTF1 (Fig. 6C). Post-mortem brain samples from 10 female patients with Alzheimer’s disease (age range: 75–90 years) and six female non-demented controls (age range: 80–87 years) were

Figure 6 Presence of ectodomain-shed, C-terminal fragment of N-cadherin (NcadCTF1) is increased in post-mortem brains of patients with Alzheimer’s disease. (A) Western blot of post-mortem brain homogenates from non-demented controls (left from dotted line) and from patients with Alzheimer’s disease (right from dotted line). Braak stage, age and post-mortem delay (PMD) of individuals (all female) are given above the corresponding lane. Post-mortem brain samples from two different sources were used: Netherlands Brain Bank (centre) and European BrainNet (lanes added on the left and right, respectively). All lanes taken from the same western blot. Blot was probed with a N-cadherin antibody (C32) against the C-terminal domain. Actin was used as loading control. (B) Densitometric quantification of the presence of NcadCTF1 (relative to actin loading control) in western blots (two independent blots analysed for each brain sample) of post-mortem brain homogenates from 10 patients with late-onset Alzheimer’s disease (AD) and from 6 non-demented individuals (controls). Values normalized to mean of control. Statistical comparison was done using the Kolmogorov-Smirnov test followed by Student’s t-test. *P < 0.05. (C) Inhibition of γ-secretase by L685,458 (5 μM) in cultured human neural stem cells (NSC) for 6 h increased the presence of ectodomain-shed, C-terminal fragment of N-cadherin (NcadCTF1) because its degradation is blocked. This effect was used for identification of NcadCTF1 in post-mortem brain homogenates of patients with Alzheimer’s disease. (D) Use of a different antibody (3B9) against the C-terminal domain of N-cadherin resulted in the identical pattern of bands in western blot, thus confirming recognition of N-cadherin fragments.
analysed (Fig. 6A). Both qualitative and quantitative analysis of the western blot bands corresponding to NcadCTF1 revealed a clearly increased presence of ectodomain-shed NcadCTF1 in the post-mortem brains of at least half of the patients with Alzheimer’s disease, while the actin loading controls did not differ between patients and non-demented controls (Fig. 6A and B). Densitometric NcadCTF1 signals normalized to actin signals were significantly different between the patient and control groups.

Discussion

We studied the consequences of inhibiting the function of the synaptic adhesion molecule N-cadherin for amyloid-β-induced synapse impairment and found a strong acceleration of the deleterious effects of amyloid-β. First, we characterized the effects of amyloid-β peptides on AMPA mEPSCs and on the overall number of synapses in cultured cortical neurons. In line with published work from several other groups (Buttini et al., 2005; Chang et al., 2006; Hsieh et al., 2006; Shankar et al., 2007; Ting et al., 2007; Evans et al., 2008; Nimmrich et al., 2008; Parodi et al., 2010), we observed a strong reduction in AMPA mEPSC frequency and a relatively slight loss of synaptic sites. The effects of amyloid-β oligomers occurred on a time scale of days in our cell culture system, and thus Alzheimer’s disease pathology appeared to be compressed in time. The relatively high concentration of amyloid-β peptides in the 7PA2 supernatant (in the nanomolar range) could explain this compression. An acceleration of amyloid-β-induced synaptic defects and Alzheimer’s disease pathology can also be observed under less severe conditions in mouse models, as exemplified by the pathology-enhancing effects of the expression of familial Alzheimer’s disease-related presenilin mutations (Borchelt et al., 1997).

Since the effect on AMPA mEPSCs was much more pronounced than the observed synapse loss, functional synaptic defects such as presynaptic changes in vesicle exo/endocytosis, downregulation of AMPA receptors and postsynaptic silencing of synapses appeared to be triggered by amyloid-β peptides. Intriguingly, inhibition of N-cadherin function strongly accelerated the deleterious effect of amyloid-β on mEPSC frequency. The pathophysiologically increased C-terminal fragment of N-cadherin also led to a strong acceleration of amyloid-β-induced synapse impairment. Both expression of NcadCTF1 and reducing its degradation by inhibiting γ-secretase activity were equally effective. As expression levels of NcadCTF1 in post-mortem brains of patients with Alzheimer’s disease were higher than in the control group, the acceleration of amyloid-β-induced synapse impairment by NcadCTF1 might contribute to progressive synapse loss in this disease.

The expression of extracellularly truncated N-cadherin fragments as a dominant-negative molecular tool to inhibit N-cadherin function has been well established (Riehl et al., 1996; Togashi et al., 2002; Bozdagi et al., 2004; Stan et al., 2010). The dominant-negative effect is caused by binding of endogenous catenins, thus leading to a mislocalization of β-catenin away from the endogeneous N-cadherin/catenin complexes (Niemann et al., 1999; Bozdagi et al., 2004). This might lead to an enhanced loss of N-cadherin from the surface membrane by endocytosis, which is tightly controlled by catenin expression (Niemann et al., 1999; Tai et al., 2007; Arrikath and Reichardt, 2008). The competitive loss of catenins might furthermore inhibit trans-synaptic N-cadherin adhesion and signalling (Murase et al., 2002; Bamji et al., 2003; Arrikath and Reichardt, 2008; Stan et al., 2010).

The inhibition of N-cadherin function by NcadCTF1 appears to reduce the overall long-term stability of synapses and might thus—in a synergistic manner—enhance the amyloid-β-triggered impairment of synapses. A synergistic action of N-cadherin inhibition and amyloid-β peptides is also supported by observations that strong N-cadherin inhibition or knockout alone leads to functional synaptic defects (Bozdagi et al., 2004; Jüngling et al., 2006; Vitureira et al., 2011). Although the exact mechanisms of action of amyloid-β peptides are still largely unknown, there are several molecular pathways that are crucially involved in amyloid-β-induced synapse impairment and are also strongly affected by a loss of N-cadherin function. Oligomeric amyloid-β peptides have been demonstrated to inhibit long-term potentiation in the hippocampus (Walsh et al., 2002; Shankar et al., 2008), possibly by shifting the balance between long-term potentiation and long-term depression to a state where the induction of long-term depression is dominant. Furthermore, amyloid-β peptides have been suggested to activate long-term depression-associated mechanisms, thus leading to synapse impairment and loss (Hsieh et al., 2006; Ting et al., 2007; Shankar et al., 2008; Li et al., 2009a; Palop and Mucke, 2010). amyloid-β-induced activation of long-term depression pathways might lead to both direct postsynaptic silencing and presynaptic impairment through retrograde signalling, ultimately resulting in a reduced AMPA mEPSC frequency. In line with the idea that N-cadherin inhibition and amyloid-β peptides act synergistically, loss of N-cadherin function has been described to inhibit long-term potentiation (Tang et al., 1998; Bozdagi et al., 2010). Although we did not obtain direct evidence for an enhancement of postsynaptic AMPA receptor downregulation (mEPSC amplitudes were unaffected by NcadCTF1), the combined action of N-cadherin inhibition and amyloid-β peptides might synergistically enhance both pre- and postsynaptic silencing of synapses. Furthermore, amyloid-β-triggered synapse impairment and synapse loss have repeatedly been shown to be influenced by NMDA receptor activity (Hsieh et al., 2006; Shankar et al., 2007, 2008; Li et al., 2009a). In addition, the synaptic expression of NMDA receptors has been described to be reduced by amyloid-β peptides (Snyder et al., 2005; Hsieh et al., 2006; Dewachter et al., 2009), thus potentially shifting synaptic NMDA receptor function from bidirectional plasticity to the induction of long-term depression-like phenomena.

In addition to interfering with long-term plasticity-related molecular pathways, amyloid-β peptides have been proposed to act on presynaptic vesicle clusters (Buttini et al., 2005; Evans et al., 2008). More specifically, amyloid-β peptides have been suggested to induce depletion of releasable vesicles, possibly by increasing intracellular calcium concentrations (Ting et al., 2007; Prillier et al., 2009; Parodi et al., 2010). Furthermore, amyloid-β peptides have been described to inhibit endocytotic recycling of synaptic vesicles (Kelly and Ferreira, 2007). In line with an
enhancement of amyloid-β-triggered depletion of presynaptic vesicles upon N-cadherin inhibition, the N-cadherin/catenin complex has repeatedly been demonstrated to play a crucial role in presynaptic vesicle clustering, vesicle replenishment at the active zone and vesicle cluster stabilization (Togashi et al., 2002; Bamji et al., 2003; Bozdagi et al., 2004; Jöngling et al., 2006; Arikath and Reichardt, 2008; Stan et al., 2010; Vitureira et al., 2011). Thus, destabilization of presynaptic vesicle clusters by inhibition of N-cadherin function might strongly facilitate amyloid-β-induced vesicle depletion, ultimately leading to accelerated synapse impairment and synapse loss.

Under in vivo conditions without over-expression, the presence of the N-cadherin function-blocking NcadCTF1 might be strongly enhanced in all physiological and pathophysiological situations in which either the expression of presenilins is reduced or the function of γ-secretase is inhibited. Interestingly, the complete knockout of presenilins in mice resulted in reduced NMDA receptor responses and reduced presynaptic vesicle release (Zhang et al., 2009, 2010). As discussed above, inhibition of N-cadherin function by non-processed NcadCTF1 might, at least in part, underlie these synaptic phenotypes. In line with the presenilin knockout studies, we show in this article that blocking presenilin function by a γ-secretase inhibitor strongly increased the presence of NcadCTF1 and led to an accelerated amyloid-β-induced impairment of synapse function. These results provide an additional mechanism, namely the inhibition of N-cadherin function, for deleterious side effects of γ-secretase inhibitors, thus further strengthening concerns about the therapeutic use of these drugs (Panza et al., 2010).

A variety of different clinical mutations in the presenilin genes, which are associated with familial Alzheimer’s disease, have been characterized (De Strooper, 2007; Selkoe and Wolfe, 2007). Most of these mutations are dominant (partial) loss-of-function mutations resulting in altered proteolytic activity, but these mutations nevertheless strongly accelerate disease progression in familial Alzheimer’s disease (Moehlmann et al., 2002; Marambaud et al., 2003; Bentahir et al., 2006; Shen and Kelleher, 2007). This has been suggested to be caused by an increased amyloid-β_{42} to amyloid-β_{40} ratio that results from altered γ-secretase activity (Borchelt et al., 1996; Moehlmann et al., 2002; Bentahir et al., 2006; Selkoe and Wolfe, 2007; Shen and Kelleher, 2007). In addition, the expression of these hypofunctional presenilin mutations has been demonstrated to lead to the enhanced presence of endogeneous γ-secretase substrates, such as NcadCTF1 (Bentahir et al., 2006). NcadCTF1 might in turn inhibit N-cadherin function as discussed above and thus might contribute to presenilin mutation-induced synapse destabilization in familial Alzheimer’s disease. In line with this hypothesis, expression of the hypofunctional L166P PS1 mutation has been shown to result in an increased level of NcadCTF1 (Bentahir et al., 2006) and to lead to a strong acceleration of amyloid-β-induced synapse impairment in our study. In summary, an increased presence of NcadCTF1 is likely to occur in familial Alzheimer’s disease caused by presenilin mutations, and this might strongly accelerate amyloid-β-triggered synapse damage by inhibiting N-cadherin function.

Analysing the post-mortem brains of patients with late-onset Alzheimer’s disease, we found strongly increased levels of NcadCTF1 in ~50% of the individuals. Thus, an acceleration of amyloid-β-triggered synapse impairment and synapse loss by synergistic effects of an inhibition of N-cadherin function appears conceivable during progression of sporadic late-onset Alzheimer’s disease. The increase in NcadCTF1 might indicate that the processing of NcadCTF1 by γ-secretase is decreased or, alternatively, that the production of NcadCTF1 by ADAM10 is increased. In conclusion, we present evidence that inhibition of N-cadherin function by an increased presence of NcadCTF1 leads to a strong acceleration of amyloid-β-triggered synapse impairment. This novel synergistic mechanism might play a role in early-onset familial Alzheimer’s disease because presenilin mutations lead to hypofunctional γ-secretase and thus to increased amyloid-β_{42} as well as enhanced NcadCTF1 levels. Most importantly, the presence of NcadCTF1 also appeared to be increased in late-onset Alzheimer’s disease, thus potentially accelerating amyloid-β-induced synapse damage as an early event in the etiology of the disease. An involvement of NcadCTF1 in synapse loss has strong implications for potential therapeutic approaches, e.g. γ-secretase modulators reducing amyloid-β_{42} production and simultaneously not inhibiting NcadCTF1 breakdown would be highly desirable.

Acknowledgements

We thank Dr S. Weggen for providing the pLPCX-PS1L166P construct, Dr R. Kemler and Dr C. Holt for providing the N-cadherin expression vectors, Dr D.J. Selkoe for providing the amyloid-β secreting 7PA2 CHO cell line and Dr R. Kemler for providing N-cadherin knockout embryonic stem cells. We also thank Martina Bohndorf for excellent technical assistance.

Funding

Deutsche Forschungsgemeinschaft [GRK1033 to K.G. and C.K.]; Volkswagenstiftung and EU-FP7 PRIORITY (to C.K.).

Supplementary material

Supplementary material is available at Brain online.

References


Kelly BL, Ferreira A. Beta-amyloid disrupted synaptic vesicle endocytosis in cultured hippocampal neurons. Neuroscience 2007; 147: 60–70.


