Aβ Oligomers Alter NMDA Receptor Composition and Function in Early Stages of Alzheimer’s Disease

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Abstract

Background

Amyloid beta (Aβ)-mediated synapse dysfunction is an early event in Alzheimer's disease (AD) pathogenesis and previous studies suggest that NMDA receptor (NMDAR) dysregulation may contribute to these pathological effects. Although Aβ peptides impair NMDAR expression and activity, the mechanisms mediating these alterations in early stages of AD are unclear. Here, we show that Aβ oligomers activate PKC, phosphorylate NR2B subunit and modulate its synaptic localization and function.

Methods

We isolated postsynaptic fractions (PSD) of AD prefrontal cortex and hippocampus of 6-month-old 3xTg-AD mice to quantify NR2B, PSD-95 and Aβ1-42 levels. To investigate the effects of Aβ oligomers on NR2B and PSD-95 expression, we use a range of techniques including mouse intrahippocampal injections of Aβ oligomers, isolation of protein membranes by cell-surface biotinylation, and synaptosomal fractionation as well as in vivo surface immunolabeling of EGFP-NR2B. Ca2+ imaging and PKC activity were monitored by fluorescent Ca2+ indicators and FRET analysis.

Results

We observed that NMDAR subunit NR2B and PSD-95 levels were aberrantly upregulated and correlated with Aβ42 load in human PSD fractions from early stages of AD patients as well as in hippocampus of 3xTg-AD mice. Importantly, NR2B and PSD95 dysregulation was revealed by an increased expression of both proteins in Aβ-injected mouse hippocampi. In cultured neurons, Aβ oligomers increased NR2B-containing NMDAR density and NMDA-induced synaptic Ca2+ influx in neuronal membranes in addition to colocalization in dendrites of NR2B subunit and PSD95. Mechanistically, Aβ oligomers required integrin β1 to promote synaptic location and function of NR2B-containing NMDARs and PSD95 by phosphorylation through classic PKCs.

Conclusions

These results provide evidence that Aβ oligomers modify the contribution of NR2B to NMDAR composition and function in early stages of AD through an integrin β1 and PKC-dependent pathway. These data reveal a novel role of Aβ oligomers in synaptic dysfunction that may be relevant to early-stage AD pathogenesis.

Full-text

Due to technical limitations, full-text HTML conversion of this manuscript could not be completed. However, the manuscript can be downloaded and accessed as a PDF.
NR2B and PSD-95 levels are elevated in prefrontal cortex from AD patients at early disease stages, and correlate with Aβ load. (A) Western blot of NR2B, PSD-95 and β-actin in PSD fraction of post-mortem prefrontal cortex from controls and AD patients at different Braak stages (Braak II-VI). (B-C) Scatter plot showing NR2B and PSD-95 levels in controls (Ctrl) and AD subjects (n = 4-7 per group). Data were analysed with one-way ANOVA followed by Bonferroni’s test; *p<0.05, **p<0.01, ***p<0.001. (D) ELISA determination of Aβ peptide levels in PSD fractions of same samples with different AD stages and controls as indicated in the scatter plot. Data were analysed with one-way ANOVA followed by Sidak’s test; **p<0.01, ***p<0.001. (E, F) Scatter plots of NR2B and PSD95 versus Aβ levels in samples of PSD fractions of human prefrontal cortex from controls and AD patients. Color codes of control and AD
samples are indicated in F. Note the significant positive correlation between NR2B or PSD95 and Aβ levels in control and AD I-III samples (p=0.0133 and p=0.0311, respectively). Data were analyzed with a linear regression method.

Figure 2

Aβ peptide load in 3xTgAD mice correlates with NR2B and PSD95. (A-D) Western blots and quantitative analysis of NR2B, PSD-95, and synaptophysin (Syn) in isolated synaptic terminals of 6-month-old control
and 3xTg-AD mice (n=5 animals per group). Scatter plots represent the means ± S.E.M. of values normalized to corresponding β-Actin; *p<0.05, **p<0.01; unpaired Student’s t test. (E) Scatter plot of Aβ peptide levels in synaptosomes of same samples as determined by ELISA. Data were analyzed with unpaired t-test; ***p<0.001. (F, G) Scatter plots of NR2B and PSD95 versus Aβ levels in synaptosomes of controls and 3xTg-AD mice. Note the significant positive correlation between NR2B or PSD95 and Aβ levels in mouse samples (p=0.036 and p=0.011, respectively). Data were analyzed with a linear regression method. (H, J) Coronal sections of mouse brains were analyzed after 7 days of vehicle or Aβ (135 ng) injection. Photomicrographs show NR2B (H) and PSD-95 (J) immunolabeling in dentate gyrus. (I, K) Scatter dot plots show the mean values of NR2B and PSD95 intensities in vehicle- and Aβ-injected mice. 2-3 brain sections of 9-16 mice were used. Data were analyzed with unpaired Student’s t-test; *p<0.05.
Aβ promotes alterations of NR2A and NR2B subunit distribution and function in neurons. (A-C) Cultured neurons were incubated with Aβ 1μM for 30 min or 24h, and total and biotinylated neuron-surface NR2A and NR2B were identified by western blot (A). Graph bars (means ± S.E.M.) represent the volume band intensities of total NMDAR subunits normalized to β-actin (B) and surface NMDA receptors normalized to total NMDARs (C). (D, E) Neurons were exposed to 1 μM Aβ for 30 minutes or 24 h, loaded with Fura-2AM for 30 minutes and Ca2+ rise was evaluated after application of 30 μM NMDA. Each recording represents the average of 52-65 cells from 3 independent experiments. (E) Violin plot represents data distribution and mean ± S.E.M. of the area under curve for each condition in arbitrary units (a.u.). Data were analyzed with one-way ANOVA followed with Dunnet´s test; **p<0.01; n=3 cultures, 191 cells in total analysis. (F) Effects of 10 μM TCN-201, an NR2A antagonist, in NMDA-induced Ca2+ influx in untreated and Aβ-treated neurons for 30 min. Violin plot shows data distribution and mean ± S.E.M. of area under curve of NMDA responses. Data were analyzed with one-way ANOVA followed by Bonferroni´s test; *p<0.05, ***p<0.001. (G) Ca2+ recordings following synaptic and extrasynaptic NMDAR activation in control and neurons treated with 1μM Aβ for 30 min (n=3 experiments; 67 and 63 cells, respectively). Synaptic NMDAR activity was recorded after addition of 50 μM bicuculline and 2.5 mM 4-AP. Extrasynaptic NMDAR-mediated ion fluxes were recorded with NMDA agonist (30 μM) following synaptic NMDAR blockade with 10 μM MK-801 applied during treatment with bicuculline. (H) Violin plot represents data distribution and mean ± S.E.M. of area under Ca2+ response curve for each condition. Data were analyzed with one-way ANOVA followed by Sidak´s test ***p<0.001.
Figure 4

Aβ treatment increases the location of NR2B subunit and PSD-95 in neuronal surface and favors their colocalization. (A) Neurons were exposed to 1 μM Aβ for 30 min, 3 h and 24 h. Total, synaptic and cytosolic protein samples were extracted and NR2B and PSD-95 levels were detected by immunoblot. (B, C) Graph bars show the means ± S.E.M of NR2B and PSD95 band volumes normalized to corresponding β-Actin of three independent experiments expressed as arbitrary units (a.u.). *p<0.05, **p<0.01 paired two-way ANOVA followed by Bonferroni’s test. (D) Neurons, expressing pEGFP-NR2B protein, were labeled in vivo using an antibody against EGFP (green), fixed in methanol and stained with anti PSD-95 in red. (E, F)
High magnification photographs and dot plot of Pearson correlation coefficient shows co-assembling of EGFP-NR2B with PSD-95 in dendrites is higher in Aβ-treated neurons. Data are represented as means ± S.E.M. of 52 ROIs from at least 3 independent experiments. Data were analyzed with paired Student’s t test *p<0.05.

Figure 5
Amyloid β promotes phosphorylation and activation of PKC which controls NR2B surface localization. (A) Images show effective targeting of Myr-Palm CKAR into neuron plasma membranes. (B-D) FRET recordings of PKC activity using Myr-Palm CKAR in neurons after application of 1 mM PMA, 500 nM Gö6983 or 1 μM Aβ plus 100 nM calyculin. Recordings are represented as means ± S.E.M. of three to five experiments. (E) Neurons were pretreated or not with 100 nM Gö6983 for 1 h and stimulated or not with 1 μM Aβ for 30 minutes. Immunoblots show pNR2B at Ser1303 in total cell extracts. Histogram represents pNR2B normalized with β-actin. Data were analyzed with two-way ANOVA followed by Bonferroni test *p<0.05. (F) Neurons were exposed to 1μM Aβ for 15, 30 and 60 min, and pPKC was examined by western blot (n=5). Histogram represents means ± S.E.M. of band volume intensities of pPKC normalized to β-actin levels. Data were analyzed with with one-way ANOVA *p<0.05. (G-I) NMDA-mediated Ca2+ responses in isolated neurons pretreated for 30 min with 1 mM PMA (G), with 1μM Aβ (H) or Aβ together with 100 nM Gö6983 (I). (J) Violin plot represents the data distribution and mean ± S.E.M. of area under Ca2+ curve for each condition expressed as arbitrary units (a.u.). Data were analyzed with one-way ANOVA followed with Dunnet’s test; **p<0.01; n=3 cultures, 262 cells. (K) Neurons were treated with 1 μM Aβ for 30 min in the presence or in the absence of 100 nM Gö6983 and NR2B subunit levels were examined by western blot in total and synaptic fractions. (L) Histogram shows quantification of synaptic NR2B in immunoblots (n=3). Data are represented as means ± S.E.M. of band volume intensities normalized to β-actin. Data were analyzed with two-way ANOVA followed by Bonferroni test *p<0.05.
Integrin β1 mediates Aβ-induced PKC activation and surface NR2B expression in primary cortical neurons. (A) PKC phosphorylation was measured by western blot in total cell extracts from neurons previously preincubated with 100 μM RGDS and stimulated with 1 μM Aβ. Histogram represents quantification of phosphorylated PKC after normalization with β-actin (n=4). Data were analyzed with one-way ANOVA *p<0.05. (B) Total cell extracts were obtained after treatment with 1 μM Aβ or 0.5 μg/ml TS2-16 for 30 minutes and PKC phosphorylation was analysed by western blot. Histogram represents quantification of phosphorylated protein after normalization with β-actin (n=4). Data were analyzed with one-way ANOVA *p<0.05, 937 **p<0.01. (C) Neurons expressing Myr-Palm-CKAR reporter were pretreated with 100 nM calyculin, 0.5 μg/ml isotype control IgM or CD29 antibody, an integrin β1 inhibitor, and Aβ-induced PKC activity was measured by FRET. Data are represented as means ± S.E.M. of three to five different experiments. (D, E) Neurons, loaded with Fura-2AM, were preincubated with 0.5 μg/ml IgM or
CD29 and exposed to 1 μM Aβ (D) or incubated with TS2-16 (E) and intracellular Ca2+ levels after 30 μM NMDA application were measured by microfluorimetry. Violin plots show data distribution and mean ± S.E.M. of area under Ca2+ curve for each condition expressed as arbitrary units (a.u.) of 70-90 cells from at least 3 experiments. Data were analyzed with paired Student`s t test *p<0.05. (F, G) Immunoblot of NR2B subunit levels in functional synaptosomes of primary cortical neurons pretreated with 0.5 μg/ml IgM or CD29 antibodies and stimulated with 1 μM Aβ (n=4). Data are represented as means ± S.E.M. of band volume intensities normalized to corresponding β-Actin. Data were analyzed with two-way ANOVA followed by Bonferroni test *p<0.05.

Supplementary Files

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- AdditionalMaterial.pdf