Amyloid β Peptide-(1–42) Induces Internalization and Degradation of β2 Adrenergic Receptors in Prefrontal Cortical Neurons

Dayong Wang1, Eunice Y. Yuen5, Yuan Zhou1, Zhen Yan5, and Yang K. Xiang4,2

Emerging evidence indicates that amyloid β peptide (Aβ) initially induces subtle alterations in synaptic function in Alzheimer disease. We have recently shown that Aβ binds to β2 adrenergic receptor (β2AR) and activates protein kinase A (PKA) signaling for glutamatergic regulation of synaptic activities. Here we show that in the cerebrums of mice expressing human familial mutant presenilin 1 and amyloid precursor protein genes, the levels of β2AR are drastically reduced. Moreover, Aβ induces internalization of transfected human β2AR in fibroblasts and endogenous β2AR in primary prefrontal cortical neurons. In fibroblasts, Aβ treatment also induces transportation of β2AR into lysosome, and prolonged Aβ treatment causes β2AR degradation. The Aβ-induced β2AR internalization requires the N terminus of the receptor containing the peptide binding sites and phosphorylation of β2AR by G protein-coupled receptor kinase, not by PKA. However, the G protein-coupled receptor kinase phosphorylation of β2AR and the receptor internalization are much slower than that induced by βAR agonist isoproterenol. The Aβ-induced β2AR internalization is also dependent on adaptor protein arrestin 3 and GTPase dynamin, but not arrestin 2. Functionally, pretreatment of primary prefrontal cortical neurons with Aβ induces desensitization of β2AR, which leads to attenuated response to subsequent stimulation with isoproterenol, including decreased AMPA receptor-mediated miniature excitatory postsynaptic currents. This study indicates that Aβ induces β2AR internalization and degradation leading to impairment of adrenergic and glutamatergic activities.

Alzheimer disease (AD)3 is a progressive neurodegenerative disorder characterized by extracellular amyloid plaques (1).

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2 To whom correspondence should be addressed: 407 S. Goodwin Ave., Urbana, IL 61801. Tel.: 217-265-9448; Fax: 217-333-1133; E-mail: kevinyx@illinois.edu.

3 The abbreviations used are: AD, Alzheimer disease; Aβ, amyloid β peptide; β2AR, β2 adrenergic receptor; MEF, mouse embryonic fibroblast; mEPSC, miniature excitatory postsynaptic current; AKAR2.2, A-kinase activity reporter; LC, locus ceruleus; GRK, G-protein coupled receptor C terminal; PFC, prefrontal cortex; JAMKct, β-adrenergic receptor kinase; ARR, arrestin; CFI, cytosolic fluorescence intensity; MFI, membrane fluorescence intensity; PS1, presenilin 1; APP, amyloid precursor protein; Con, control; Tricine, N-[2-hydroxy-1,1-bis(hydroxymethyl)methyl]glycine; ANOVA, analysis of variance; β2NT, N terminus of β2AR.
ing selectivity via inducing differential conformational changes and activation of specific signaling pathways (18–23). Because of the heterogeneous binding property, it is necessary to analyze the action of Aβ/H9252 on β2AR trafficking and cell function. In addition, Aβ/H9252 is not catabolized as quickly as neurotransmitters at synapses, and the concentrations of the peptide are elevated in AD brains; therefore, persistent effects of Aβ on β2AR functions are of pertinence to AD pathology.

PKA, a signaling component downstream to β2AR, promotes the function of AMPA receptors by phosphorylating AMPA receptor subunit 1 (GluR1) (24–26). AMPA receptors are ligand-gated ion channels and are one of major mediators of excitatory neurotransmission. We have shown that acute treatment with Aβ increases β2AR- and PKA-dependent phosphorylation of GluR1 and frequency and amplitude of miniature excitatory postsynaptic currents (mEPSCs) mediated by AMPA.

**FIGURE 1. Aβ induces β2AR degradation in vivo and in vitro.** A, the endogenous β2AR in cerebral tissues from wild type (WT) and presenilin 1/amyloid precursor protein (PS1/APP) double transgenic mice were examined with Western blot and quantified. n = 6; **, p < 0.01, versus control by unpaired t test. IP, immunoprecipitate. B, HEK293 cells expressing FLAG-tagged human β2AR were stimulated with either Aβ (10⁻⁶ M) or isoproterenol (Iso, 10⁻⁷ M) for 4 h. The remaining biotin-labeled β2ARs were isolated and blotted, and the Western blots (WB) were quantified. n = 6; **, p < 0.01 versus control by one-way ANOVA. C, HEK293 cells expressing FLAG-β2AR were stimulated with Aβ (10⁻⁶ M) or isoproterenol (10⁻⁷ M) for 40 min, and cells were costained with anti-FLAG and anti-Lamp I antibodies.
Here we hypothesize that a persistent treatment with Aβ/H9252, in contrast to its acute effects, may impair cell function by decreasing A2AR agonist-induced phosphorylation of GluR1, which is modulated by a GluR1-A2AR complex (3, 26, 27). In this study a persistent treatment with Aβ/H9252 indeed induces A2AR internalization and degradation leading to decreased mEPSCs mediated by AMPA receptors. Moreover, in AD animals, A2AR levels in cerebral tissues are significantly reduced, indicating its implication in brain adrenergic and glutamatergic dysfunction in AD.

EXPERIMENTAL PROCEDURES

Animals—Wild type, β1AR, β2AR, both β1AR and β2AR gene-knock-out (KO), and presenilin 1/amyloid precursor protein (PS1/APP) transgenic mice were used (28, 29). PS1/APP-transgenic mice overexpress both the human PS1 gene harboring two familial AD mutations, M146L and L286V, and the APP gene (695) with Swedish (K670N, M671L), Florida (I716V), and London (V717I) familial AD mutations. Wild type and PS1/APP transgenic mice (7 months old) were used for analysis of endogenous A2AR levels in the cerebrum. All animal experimental procedures were approved by the University of Illinois Animal Care and Use Committee.

Cell Culture and Aβ Treatment—Newborn WT, β1AR-KO, β2AR-KO, and β1/β2AR-KO mice were used to isolate PFC neurons under a stereomicroscope (30). Isolated neurons were plated on poly-D-lysine-coated dishes at a density of 1.0 × 10^6–10^7 cells/ml in DMEM/F-12 (1:1) medium containing 10% FBS, 1% insulin-transferin-sodium selenite supplement, 25 ng/ml nerve growth factor, 1 mM glutamine, 20 mM water-soluble progesterone, and 100 nM putrescine. The next day medium was changed to Neurobasal-A with B-27 supplement. Cytosine β-d-arabinofuranoside (2.5 μM, Sigma) was added 3 days after plating. Neurons were cultured for 2–3 weeks before experiments. HEK293 cells, wild type mouse embryonic fibroblasts (MEFs), and MEFs lacking either arrestin 2 (ARR2) or arrestin 3 (ARR3) gene were cultured in DMEM medium containing 10% FBS and antibiotics. Aβ1–42 and amino acid sequence-inverted peptide Aβ42–1 (Biopeptide) were dissolved at 10^{-3} M in dimethyl sulfoxide (DMSO) as previously described (31), which yields primarily monomers, dimers, and trimmers with low levels of oligomers. Cells were treated with soluble Aβ1–42 peptide as indicated. Our previous study shows that under these conditions Aβ1–42 dimers are the primary species bound to human FLAG-β2AR expressed on the plasma membrane (3).

cDNA Constructs—Human FLAG-β2AR and human FLAG-β1AR are gifts from Dr. Randy Hall (Emory University, GA). The GRK3A mutant β2AR lacking three GRK phosphorylation sites and the PKA4A mutant β2AR lacking four PKA phosphorylation sites, GFP-ARR3 and V54D dominant negative GFP-ARR3, HA-dynamin, and K44E dominant negative HA-dynamin, and the C terminus of GRK2 (also named β-adrenergic receptor kinase C-terminal (βARKct)) were constructed as described previously (32–34).

Immunofluorescence Microscopy—FLAG-tagged wild type, GRK3A mutant, or PKA4A mutant β2AR was expressed in HEK293 cells or MEFs by transfection or in freshly isolated PFC neurons (3). Here we hypothesize that a persistent treatment with Aβ, in contrast to its acute effects, may impair cell function by decreasing β2AR agonist-induced phosphorylation of GluR1, which is modulated by a GluR1-β2AR complex (3, 26, 27). In this study a persistent treatment with Aβ indeed induces β2AR internalization and degradation leading to decreased mEPSCs mediated by AMPA receptors. Moreover, in AD animals, β2AR levels in cerebral tissues are significantly reduced, indicating its implication in brain adrenergic and glutamatergic dysfunction in AD.
neurons by virus infection at 100 multiplicity of infection. After a 2-day expression, cells were treated with drugs for detection with anti-FLAG M1 (Sigma) or anti-β2AR antibody (Santa Cruz Biotechnology). Wild type and K44E-mutant dynamin were detected with anti-HA antibody (Covance). Lysosomes were stained by anti-Lamp I antibody (BD Pharmingen). Alexa488- and Alexa594-conjugated secondary antibodies (Invitrogen) were used to reveal the primary antibodies. Wild type and V54D-mutant ARR3 and ARKct were indicated by GFP fluorescence. Images were quantified with ImageJ-based Fiji open source image-processing package. Briefly, images were rotated to enable optimal selection of areas across the cell body. Plot profile analysis was applied to selected regions for measure of fluorescence intensity. Average of cytosolic fluorescence intensity (CFI) of β2AR was divided by that of membrane fluorescence intensity (MFI) for analysis of β2AR internalization. The ratio of CFI/MFI after stimulation was normalized against the ratio of CFI/MFI at resting state.

Receptor Degradation Assay—After serum starvation, cell surface proteins were labeled with 300 μg/ml biotin (Pierce) in PBS with calcium and magnesium containing 10 mM glucose (PBS/G) at 4 °C for 30 min. Free biotin was quenched with 100 mM glycine in PBS/G for 30 min followed by washing with ice-cold PBS/G. Cells were treated for 4 h with medicines replenished every 30 min. Cells were lysed in ice-cold lysis buffer (25 mM HEPES, pH 7.5 at 4 °C, 10% glycerol, 45 mM NaCl, 2 mM Na2VO4, 5 mM EDTA, 5 mM EGTA, 50 mM NaF, 1% Triton X-100, 8 mM NaN3, and protease and phosphatase inhibitor

**FIGURE 3.** Aβ induces internalization of β2AR but not β1AR or chimeric β1NT-β2AR. A, shown is distribution of FLAG-β2AR, FLAG-β1AR, and FLAG-β1NT-β2AR, in which the N terminus of the receptor was replaced by that of β1AR in HEK293 cells after Aβ (10−6 M) or isoproterenol (Iso, 10−7 M) treatment. B, shown are representative diagrams of fluorescence intensity in the selected regions of individual cells expressing FLAG-β2AR, indicated by the yellow frames in panel A; the cell membrane is indicated by arrows. C, Aβ-induced internalization of β2AR, β1AR, or chimeric β1NT-β2AR was quantified with Fiji image-processing package. n = 16; *, p < 0.05; **, p < 0.01 versus control by one-way ANOVA.
Aβ induces β2AR Internalization

Images in both channels were subjected to background subtraction, and ratios of yellow-to-cyan color were calculated at different time points. In some experiments PFC neurons were pretreated with Aβ (10^{-6} M) or isoproterenol (Iso, 10^{-7} M) for 40 min, and the distribution of β2AR was examined with immunofluorescence staining. B, the images of Aβ-induced internalization of β2AR in panel A were quantified. n = 16; **, p < 0.01 versus control by one-way ANOVA.

FIGURE 4. Aβ-induced β2AR internalization requires GTPase dynamin. A, FLAG-β2AR was expressed together with either wild type or K44E dominant negative dynamin in HEK293 cells. Cells were stimulated with Aβ (10^{-6} M) or isoproterenol (Iso, 10^{-7} M) for 40 min, and neurons were then washed 3 times before administration of isoproterenol (10^{-7} M).

Whole-cell Recording in Cultured PFC Neurons—The AMPA receptor-mediated mEPSCs in rat PFC cultures were recorded as described previously (41). The internal solution consisted of 130 mM cesium methanesulfonate, 10 mM CsCl, 4 mM NaCl, 10 mM HEPES, 1 mM MgCl2, 5 mM EGTA, 2.2 mM QX-314, 12 mM phosphocreatine, 5 mM MgATP, 0.5 mM Na2GTP, 0.1 mM leupeptin, pH 7.2–7.3, 265–270 mosm. The external solution consisted of 127 mM NaCl, 5 mM KCl, 2 mM MgCl2, 2 mM CaCl2, 12 mM glucose, 10 mM HEPES, 1 mM tetrodotoxin, 5 μM bicuscul-
ine, pH 7.3–7.4, 300–305 mosM. Recording was conducted with a holding potential of \(-70 \text{ mV}\) in the presence of 25 \(\mu\text{M}\) d-2-amino-5-phosphonovalerate to block NMDA receptor-mediated components of mEPSCs. Neurons were treated with 0.1% DMSO or \(\text{A}^{\beta}\) (10\(^{-6}\) M) for 40 min followed by 3 times washing with 0.1% DMSO-containing media. Synaptic currents were analyzed with Mini Analysis Program (Synaptosoft, Leonia, NJ).

**Statistical Analyses**—Unpaired \(t\) tests and one- or two-way ANOVA were used to compare different groups with Prism software as indicated (Graphpad). Statistical comparisons of the amplitude and frequency of synaptic currents (mean \pm S.E.) were made using the Kolmogorov-Smirnov test. A \(p\) value less than 0.05 is considered significant.

**RESULTS**

During the early development of AD, the central noradrenergic system undergoes substantial changes in response to the degeneration of its main site, the LC. Recent publications have shown that an acute treatment with \(\text{A}^{\beta}\) initiates signal transduction via \(\beta_2\text{AR}\) as an allosteric partial agonist (3, 16, 17). In this study we sought to understand the impact of a prolonged treatment with \(\text{A}^{\beta}\) (10\(^{-6}\) M) on protein levels and function of \(\beta_2\text{AR}\) in vivo and in vitro. We first examined \(\beta_2\text{AR}\) protein levels in the cerebrums of 7-month-old PS1/APP double transgenic mice. The \(\beta_2\text{AR}\) levels were significantly lower than those in wild type animals (Fig. 1A). In agreement, human \(\beta_2\text{AR}\) expressed in HEK293 cells underwent significant degradation after 4 h of treatment with \(\text{A}^{\beta}\) (10\(^{-6}\) M) (Fig. 1B). Accordingly, a 40-min stimulation of human \(\beta_2\text{AR}\) internalization and degradation occurs after a prolonged treatment with \(\text{A}^{\beta}\). We then set out to examine whether \(\text{A}^{\beta}\) induces \(\beta_2\text{AR}\) internalization in HEK293 cells. We first examined \(\beta_2\text{AR}\) internalization in panel A were quantified. \(n = 16; **, \ p < 0.01\) versus control by one-way ANOVA.

**FIGURE 5.** \(\text{GRK}, \text{not PKA}, \) phosphorylation of \(\beta_2\text{AR}\) is required for \(\text{A}^{\beta}\)-induced receptor internalization. A, distribution of PKA and GRK phosphorylation site-mutant \(\beta_2\text{ARs}\) (PKA4A \(\beta_2\text{AR} and GRK3A \(\beta_2\text{AR}\)) after stimulation with \(\text{A}^{\beta}\) (10\(^{-6}\) M) or isoproterenol (Iso, 10\(^{-7}\) M) for 40 min in HEK293 cells is shown. B, the images of \(\text{A}^{\beta}\)-induced internalization of \(\beta_2\text{AR}\) in panel A were quantified. \(n = 16; **, \ p < 0.01\) versus control by one-way ANOVA. C Effect of a membrane-permeable PKA inhibitor, myristoylated-PKI, on \(\text{A}^{\beta}\)-induced \(\beta_2\text{AR}\) internalization. Cells were treated with \(\text{A}^{\beta}\) (10\(^{-6}\) M), isoproterenol (10\(^{-7}\) M), and PKI (10\(^{-5}\) M) for 40 min, and the distribution of the receptor was examined. D, the images of \(\text{A}^{\beta}\)-induced internalization of \(\beta_2\text{AR}\) in panel C were quantified. \(n = 16; **, \ p < 0.01\) versus control by one-way ANOVA.
Aβ induces β2AR Internalization

A. β2AR internalization was induced by Aβ treatment (10^{-6} M) in HEK293 cells. B. β2AR internalization was induced by Aβ treatment (10^{-6} M) in HEK293 cells. C. β2AR internalization was induced by Aβ treatment (10^{-6} M) in HEK293 cells. D. β2AR internalization was induced by Aβ treatment (10^{-6} M) in HEK293 cells. E. β2AR internalization was induced by Aβ treatment (10^{-6} M) in HEK293 cells.

We then examined factors governing Aβ-induced β2AR trafficking. First, we examined whether β2AR internalization induced by Aβ is dependent on dynamin GTPase activity. Overexpression of K44E dominant negative dynamin, but not wild type dynamin, almost completely inhibited β2AR internalization in HEK293 cells under either Aβ (10^{-6} M) or isoproterenol (10^{-7} M) stimulation (Fig. 4, A and B). This suggests that these two ligands induce receptor internalization via similar endocytosis pathways. PKA and GRK can phosphorylate β2AR at different sites, which are engaged in dynamin- and arrestin-dependent G protein-coupled receptor desensitization and trafficking. In order to know which kinase is involved in the Aβ-induced β2AR internalization, PKA and GRK phosphorylation site-mutant β2ARs, termed PKA4Aβ2AR and GRK3Aβ2AR respectively, were expressed in HEK293 cells. Immunofluorescence staining showed that mutant β2ARs lacking PKA phosphorylation sites, not GRK phosphorylation sites, were internalized upon Aβ (10^{-6} M) treatment (Fig. 5, A and B). Inhibition of PKA activity by a specific inhibitor, PKI (10^{-5} M),
did not block the Aβ-induced β2AR internalization (Fig. 5, C and D). βARKct, which inhibits GRK2-mediated phosphorylation of β2AR, blocked the Aβ-induced β2AR internalization (Fig. 6, A and B). Consistently, Aβ (10⁻⁶ M), but not amino sequence-inverted Aβ (iAβ, 10⁻⁶ M), induced a dose- and time-dependent increase in GRK-dependent phosphorylation of β2AR (Fig. 6, C–E). The GRK-mediated phosphorylation of β2AR occurred at slower kinetics, consistent with the observed slower internalization rate induced by Aβ. Next, ARR2 and ARR3 are non-visual arrestins and are involved in G protein-coupled receptor endocytosis. We found that Aβ (10⁻⁶ M) and isoproterenol (10⁻⁷ M) induced β2AR internalization in wild type and ARR2-deficient MEF cells but not in ARR3-deficient cells (Fig. 7, A and B). Moreover, Aβ (10⁻⁶ M) induced β2AR internalization in HEK293 cells expressing wild type ARR3 but not V54D dominant negative ARR3 (Fig. 7, C and D). Together, these data indicate that Aβ (10⁻⁶ M) induces GRK2-, ARR3- and dynamin-dependent internalization, although the peptide effects are slower and less potent than those of isoproterenol (10⁻⁷ M). Furthermore, we found that Aβ (10⁻⁶ M) treatment increases the processing of APP to yield Aβ (supplemental Fig. 2), consistent with the recent study showing that agonist-induced β2AR endocytosis enhances γ-secretase activity for APP processing in lysosomes (35).

To know whether the Aβ-induced β2AR internalization impairs neuronal function, we isolated PFC neurons from neonatal mice. A 40-min treatment with Aβ (10⁻⁶ M) induced significant internalization of endogenous β2AR, not β1AR (Fig. 8A), which was quantified by the biotinylation method (Fig. 8B). Aβ (10⁻⁶ M)-induced β2AR internalization was even more evi-
Aβ induces β2AR Internalization

![Graph showing Aβ induces internalization of endogenous β2AR and transfected human β2AR, not β1AR in PFC neurons.](image)

**FIGURE 8. Aβ induces internalization of endogenous β2AR and transfected human β2AR, not β1AR in PFC neurons.** A. shown is distribution of endogenous β2AR or β1AR in wild type PFC neurons and overexpressed FLAG-β2AR in β1/β2AR-KO PFC neurons after 40 min of treatment with Aβ (10⁻⁶ M) or isoproterenol (Iso, 10⁻⁷ M). B. Aβ-induced internalization of endogenous β2AR in PFC neurons was quantified by cell surface biotinylation. n = 6. *, p < 0.05 versus control by one-way ANOVA.

dent with human β2AR overexpressed in β1AR/β2AR double-KO PFC neurons (Fig. 8A). Next, we investigated β2AR-mediated G protein-dependent signal transduction in PFC neurons. A 40-min pretreatment with Aβ (10⁻⁶ M) decreased isoproterenol (10⁻⁷ M)-induced increase in the second messenger cAMP level in the presence of β2AR antagonist CGP 20217A (10⁻⁵ M) (Fig. 9A). We also measured PKA activity induced by isoproterenol (10⁻⁷ M) in live PFC neurons with a FRET-based PKA biosensor AKAR2.2 (Fig. 9, B–F (28, 40)). Isoproterenol (10⁻⁷ M) itself induced a significant increase in PKA activity in wild type neurons (Fig. 9, C–F). However, pretreatment with Aβ (10⁻⁶ M) dramatically reduced the increase in PKA activity in response to subsequent isoproterenol stimulation. The remaining activity in wild type cells is in part due to activation of β1AR, which does not bind to Aβ (3). β2AR-KO neurons were then used to isolate the β2AR-specific effect on PKA activity. As expected, although isoproterenol (10⁻⁷ M) itself induced a significant increase in PKA activity in β2AR-KO neurons, pretreatment with Aβ (10⁻⁶ M) completely abolished the increase in PKA activity (Fig. 9, C–F). In contrast, Aβ pretreatment did not affect the isoproterenol (10⁻⁷ M)-induced increase in PKA activity in β2AR-KO neurons (Fig. 9, D and F).

Moreover, in PFC neurons cultured in the presence of β1AR antagonist CGP 20217A, pretreatment with Aβ (10⁻⁶ M) also decreased isoproterenol (10⁻⁷ M)-induced increase in PKA-mediated phosphorylation of AMPA receptor subunit GluR1 (Fig. 9G). In addition, GluR1 and β2AR form a postsynaptic protein complex in neurons (3, 26, 27), and we found that Aβ (10⁻⁶ M) also induced internalization of GluR1 in PFC neurons (supplemental Fig. 3). We also investigated isoproterenol-induced PKA-dependent mEPSCs mediated by AMPA receptors in PFC neurons after pretreatment with Aβ (Fig. 9, H–L). Isoproterenol (10⁻⁷ M) itself induced a significant increase in mEPSC amplitude (Fig. 9, H and K) and a small but not significant increase in mEPSC frequency in PFC neurons (Fig. 9, I and L). However, a 40-min pretreatment with Aβ (10⁻⁶ M) abolished isoproterenol-induced increases in AMPA receptor-mediated mEPSCs (Fig. 9, H–L).

**DISCUSSION**

Emerging evidence indicates that Aβ may induce subtle cellular dysfunction in synaptic plasticity contributing to early memory loss that precedes neuronal degeneration (42). Previous publications show that Aβ binds to β2AR and acutely activates the receptor-mediated signal transduction in both astrocytes and neurons (3, 16, 17). In this study we find that prolonged pretreatment with Aβ not only induces β2AR internalization and degradation but also decreases intracellular cAMP and PKA response to subsequently administered isoproterenol in primary PFC neurons. Because Aβ is not catabolized or cleared as quickly as catecholamines, it is conceivable that an elevated level of Aβ induces persistent β2AR internalization and degradation in AD. Accordingly, we found that protein levels of β2AR in the cerebros of PS1/APP double transgenic animals are much lower than those in wild type animals. These data indicate that prolonged elevation of Aβ levels alters postsynaptic responses to neurotransmitters via decreasing β2AR levels on the plasma membrane. On the other hand, under
chronic Aβ stimulation, persistent cAMP signaling leads to elevated PKA-dependent phosphorylation of its substrates such as GluR1 (3). The elevated basal phosphorylation of GluR1 would likely contribute to the hyperactivities of neurons for imbalanced neuronal network and functions (43), and it would also impair the response to further noradrenergic stimulation under physiological condition.

Consistently, a 40-min pretreatment with Aβ significantly decreases PKA-dependent phosphorylation of GluR1 at Ser-845 in response to subsequently administered isoproterenol and, as expected, decreases AMPA receptor-mediated mEPSCs in PFC neurons (Fig. 9). This is in contrast to the stimulatory effect on AMPA receptor activity by acute Aβ treatment (3, 24–26). In addition, GluR1, which exists with β2AR in a post-synaptic protein complex, is also internalized after prolonged Aβ stimulation, consistent with previous observations showing the removal of AMPA receptor from synapse (44). Therefore, this study suggests that a prolonged presence of Aβ in AD brain has dual effects on β-adrenergic and glutamatergic neurotransmission at synapses. First, the Aβ-induced β2AR signal leads to...
Aβ induces β2AR Internalization

elevated basal phosphorylation of AMPA receptor (3) and contributes to hyperactivities of neurons in AD animal models (43). Second, the chronic stimulation also leads to internalization of both receptors and degradation of β2AR and impairs β-adrenergic and glutamatergic neurotransmission at synapses. This study also shows that the βAR antagonist, timolol, blocks β2AR internalization. Therefore, βAR antagonism may be beneficial in AD patients, as indicated in clinical studies (45).

In the early phase of AD, the central noradrenergic system undergoes substantial changes in response to the degeneration of its main site, the LC, as well as alteration of adrenergic receptor expression in different brain regions including cortical and limbic projection areas (14). LC degeneration results in decreased noradrenaline levels in certain brain regions of AD patients (13). The reduction of LC neurons correlates with Aβ plaques, neurofibrillary tangles, and the severity of dementia (46). However, norepinephrine levels in cerebrospinal fluids of AD patients are not generally reduced and in some cases are even increased (47, 48). This is probably due to a compensatory sprouting by surviving LC neurons (49). A chronic activation of β2AR on neurons in brain areas, including the LC, may contribute to neuronal degeneration due to its regulation on activities of L-type calcium channels and AMPA receptors (3, 26, 27).

This paper, on the other hand, suggests a mechanism that Aβ induces subtle alterations in synaptic functions. Besides, β2AR and its signaling, as an integral part of the central noradrenergic system, is involved in multiple cell functions, including cell differentiation, proliferation, ion channel function, and immunomodulatory activity of cells in the brain (27, 56–58). Moreover, β2AR and AMPA receptor are critical in normal brain functions. Thus, β2AR internalization and degradation induced by Aβ could be generally implicated in various diseases with high levels of Aβ including AD, Down syndrome, and Parkinson disease (59, 60).

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