β₂ Adrenergic Receptor, Protein Kinase A (PKA) and c-Jun N-terminal Kinase (JNK) Signaling Pathways Mediate Tau Pathology in Alzheimer Disease Models*

Dayong Wang†, Qin Fu‡,1, Yuan Zhou‡, Bing Xu‡, Qian Shi§,††, Benedict Igwe§, Lucas Matt§, Johannes W. Hell§, Elena V. Wisely†, Salvatore Oddo†, and Yang K. Xiang†,‡,‡‡

From the †Department of Molecular and Integrative Physiology, School of Molecular and Cellular Biology, University of Illinois at Urbana-Champaign, Urbana, Illinois 61801, the ‡Department of Pharmacology, University of California at Davis, Davis, California 95616, the §Department of Pharmacology, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, Hubei 430030, China, and the †Department of Physiology, University of Texas Health Science Center, San Antonio, Texas 78229

Background: Accumulating evidence indicates that β receptors (BAR) may be involved in Alzheimer disease (AD) pathology and that amyloid β peptide (Aβ) may interact with β₂AR independently of presynaptic activities.

Results: β₂AR, PKA, and JNK mediate Aβ-induced phosphorylation of tau in vivo and in vitro.

Significance: This work indicates a potential mechanism for altering AD pathology by blocking β₂ARs.

Alzheimer disease (AD) is characterized by neurodegeneration marked by loss of synapses and spines associated with hyperphosphorylation of tau protein. Accumulating amyloid β peptide (Aβ) in brain is linked to neurofibrillary tangles composed of hyperphosphorylated tau in AD. Here, we identify β₂-adrenergic receptor (β₂AR) that mediates Aβ-induced tau pathology. In the prefrontal cortex (PFC) of 1-year-old transgenic mice with human familial mutant genes of presenilin 1 and amyloid precursor protein (PS1/APP), the phosphorylation of tau at Ser-214 Ser-262 and Thr-181, and the protein kinases including JNK, GSK3β, and Ca²⁺/calmodulin-dependent protein kinase II is increased significantly. Deletion of the β₂AR gene in PS1/APP mice greatly decreases the phosphorylation of these proteins. Further analysis reveals that in primary PFC neurons, Aβ signals through a β₂AR-PKA-JNK pathway, which is responsible for most of the phosphorylation of tau at Ser-214 and Ser-262 and a significant portion of phosphorylation at Thr-181. Aβ also induces a β₂AR-dependent arrestin-ERK1/2 activity that does not participate in phosphorylation of tau. However, inhibition of the activity of MEK, an upstream enzyme of ERK1/2, partially blocks Aβ-induced tau phosphorylation at Thr-181. The density of dendritic spines and synapses is decreased in the deep layer of the PFC of 1-year-old PS1/APP mice, and the mice exhibit impairment of learning and memory in a novel object recognition paradigm. Deletion of the β₂AR gene ameliorates pathological effects in these senile PS1/APP mice. The study indicates that β₂AR may represent a potential therapeutic target for preventing the development of AD.

Neurofibrillary tangles composed of hyperphosphorylated tau in the brain is a hallmark of Alzheimer disease (AD)2, and the phosphorylation of tau may be a major pathological cause of the disorder by inducing synapse loss (1–4). Increasing evidence suggests that soluble amyloid β peptide (Aβ) is linked to hyperphosphorylation of tau at serine and threonine residues (5, 6). A recent study has demonstrated that Aβ causes tau to wander into dendrites, leading to loss of synapses, spines, and microtubules (7–9). In 3xTg-AD mice harboring a knockin mutation for presenilin 1 (PS1, M146V) and transgenes for amyloid precursor protein (APPsw) and tau (taup301L), spine loss occurs exclusively at dystrophic dendrites that accumulate both Aβ oligomers and hyperphosphorylated tau intracellularly (10), and it is the phosphorylation of tau that causes the protein to stray (11). Previous publications have shown that Aβ induces phosphorylation of tau at serine and threonine residues via a myriad of signaling cascades. However, little is known about how Aβ induces tau hyperphosphorylation and AD development.

In a recent epidemiological study, it was found that antihypertensive medication, including β blockers, may reduce the risk of AD (12). Another survey in AD patients indicates that β blockers may be associated with a delay of functional decline in the patients (13). There is also evidence that β₂AR may be involved in AD pathogenesis through effects on Aβ production and inflammation (14, 15). Another study has shown recently that polymorphism in β₂AR contributes to sporadic late-onset AD, which may be related to the availability and response of β₂AR (13, 16). Meanwhile, β₂AR also plays an important role in cognition and stress-related behaviors (17, 18).

Recent studies have characterized that Aβ induces activation of β₂AR-mediated PKA- and G protein-coupled receptor

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† To whom correspondence should be addressed: Department of Pharmacology, University of California at Davis, Davis, CA 95616. E-mail: ykxiang@ucdavis.edu.

1 The abbreviations used are: AD, Alzheimer disease; Aβ, amyloid β peptide; β₂AR, β₂-adrenergic receptor; PKA, protein kinase A; Epac, exchange protein activated by cAMP; APP, amyloid precursor protein; PFC, prefrontal cortex.
kinase/arrestin-dependent signal transduction, which is presynaptic activity-independent and requires the N terminus of β₂AR (19–22). Although a prolonged treatment with Aβ induces GRK/arrestin 3-dependent internalization and degradation of β₂AR, which impairs presynaptic activity-dependent neurotransmission, the intracellular levels of cAMP and PKA activity are partially preserved, reaching a balance between receptor activation and degradation (23). Besides PKA, the internalization-associated arrestin signaling can trigger the phosphorylation of MAPK and JNK that may phosphorylate tau (24), and the activation of the exchange protein activated by cAMP (Epac) may also mediate JNK phosphorylation linked to tau (25). In this study, we aim to understand the significance of β₂AR signaling cascades in tau pathology in AD.

**EXPERIMENTAL PROCEDURES**

*Animals*—Wild-type, β₂AR knockout (β₂AR-KO), arrestin-2 knockout (arrestin-2-KO), arrestin-3 knockout (arrestin-3-KO), and presenilin 1/amyloid precursor protein double-transgenic (PS1/APP) and β₂-KO/PS1/APP mice in a B6 background were described previously (22, 23, 26). PS1/APP mice were purchased from The Jackson Laboratory (stock number 006554). They overexpress both amyloid precursor protein (695) with Swedish (K670N, M671L), Florida (I716V), and London (V717I) familial AD mutations and human presenilin 1 gene harboring two-familial AD mutations, M146L and L246V (26). PS1/APP mice were cross-bred with β₂-KO mice to produce β₂-KO/PS1/APP mice. Wild-type and transgenic mice (6 months old and 1 year old) were used for tissue and behavioral studies. All animal experimental procedures were approved by the University of Illinois Animal Care and Use Committee.

*Cell Culture and Aβ Treatment*—Newborn wild-type and knockout mice were used to isolate prefrontal cortex (PFC) neurons under a stereomicroscope (22). Isolated neurons were plated on poly-D-lysine-coated dishes at a density of 1.0 × 10³ cells/ml in DMEM/F12 medium (1:1) containing 10% FBS, growth factor, 1 mM glutamine, 20 nM water-soluble progesterone, and 100 nM putrescine. Three days later, cells were changed to serum-free neurobasal/B-27 medium containing cytosine β-d-arabinofuranoside (2.5 μM, Sigma). Neurons were cultured for 2–3 weeks before the experiment. Aβ₁₋₄₂ (Biopeptide, CA) stock solutions were made by dissolving Aβ₁₋₄₂ at 10⁻³ M in 5% ammonium hydroxide and freshly diluting in dimethyl sulfoxide just before use, which yields mostly monomers, dimers, and trimers with a small amount of higher-order oligomers (22). Cells were treated with soluble Aβ as indicated. In some experiments, inhibitors for kinases and receptors were added as indicated 10 min before administration of Aβ.

*Golgi Staining*—An FD Rapid GolgiStain™ kit (MTR Scientific, MD) was used to stain dendritic spines of neurons in the deep layer of the PFC of 1-year-old and 6-month-old wild-type, β₂-KO, PS1/APP, and β₂-KO/PS1/APP mice. Briefly, neurons were perfused with heparinized PBS and 2% paraformaldehyde, followed by an additional perfusion with PBS to wash away excessive PFA in the body. Brains were dissected out and stained with Golgi-Cox impregnation solutions. After staining, the brains were sliced at a thickness of 240 μm on a LEICA Vibratome 1000. The slices were dehydrated and mounted on slides. Images were taken using a Carl Zeiss LSM-700 microscope equipped with DIC objective lenses. All slices observable along 100-μm dendritic segments at least 25 μm from the cell soma were counted.

*Immunofluorescence Microscopy*—Wild-type, β₁-KO, PS1/APP, and β₁-KO/PS1/APP were perfused consecutively in vivo with heparinized PBS and 2% PFA. The brains were dissected out and post-fixed with 2% PFA overnight. After serial dehydration in sucrose, the brains were frozen in Tissue-Tek O.C.T compound (VWR LabShop, IL), and slices were cut at a thickness of 40 μm on a CM3050 S cryostat (Leica Microsystems, Inc., Germany). Brain slices and fixed primary neurons were blocked and permeabilized with goat serum and Nonidet P-40 in PBS and then incubated with primary antibodies. Alexa Fluor 488- or Alexa Fluor 568-conjugated secondary antibodies (Invitrogen) were used to reveal the primary antibodies. Nuclei were counterstained with DAPI (Thermo Scientific, IL). Quantification of synapsin I positively stained synapses was performed with the Analyze Particles commands of the Fiji software.

*Western Blotting*—Proteins resolved by SDS-PAGE were transferred to nitrocellulose membranes (Millipore, MA) and blocked with 5% milk in buffer (10 mM Tris–HCl (pH 7.4), 100 mM NaCl, 25 mM NaF, 8 mM NaN₃, and 0.1% Tween 20). Then the membranes were incubated with primary antibodies against phospho-tau (phospho-Ser-214 and phospho-Ser-262, Santa Cruz Biotechnology, Inc. and Invitrogen, respectively, and phospho-Thr–181, Abcam, MA) and tau (Sigma-Aldrich, MO); phospho- and total stress-activated protein kinase/JNK, GSK3α/β, Ca²⁺/calmodulin-dependent protein kinase II, and ERK1/2 (Cell Signaling Technology, Inc.); γ-tubulin (Sigma-Aldrich); or synapsin I (Cell Signaling Technology, Inc.) at 4 °C overnight. Phospho-tau antibodies recognize epitopes of phosphorylated tau of both human and mouse. After washing, membranes were incubated with secondary antibodies for detection with the Li-Cor system (Li-Cor, NE). The optical density of the bands was analyzed with the gel analyzer of the Fiji software.

*Novel Object Recognition Test*—The task was carried out according to previous publications (27, 28). The experimental apparatus consisted of a Plexiglas open-field box (40 × 40 × 29 cm). The apparatus was placed in a sound-isolated room. The novel object recognition task procedure consisted of three sessions: habituation, training, and retention sessions. Each mouse was habituated individually to the box with 10 min of exploration in the absence of objects. During the training session in the next day, two objects (A and B) were placed in the back corner of the box, 10 cm from the side wall. A mouse was then placed in the middle front of the box, and the total time spent in exploring the two objects was recorded for 10 min by the experimenter with two stopwatches. Exploration of an object was defined as directing the nose to an object at a distance of less than 2 cm and/or touching it with the nose. During the retention session on the third day (24 h after the training session), the animals were placed back into the same box, in which one of the familiar objects was replaced by a novel object, C. The animals were then allowed to explore freely for 10 min, and the time spent exploring each object was recorded. Throughout the
experiments, the objects were used in a counterbalanced manner in terms of their physical complexity and emotional neutrality. A preference index, which is the ratio of the amount of time spent in exploration of any one of the two objects (training session) or the novel object (retention session) over the total time spent exploring both objects, was used to measure cognition.

Statistical Analyses—Unpaired Student’s t test and one- or two-way analysis of variance was used to compare different groups with Prism software as indicated (GraphPad, CA). \( p < 0.05 \) was considered significant.

RESULTS

To explore the role of \( \beta_2 \)-AR signaling in tau pathology in relationship with A\( \beta \) in AD, we cross-bred the AD animal model overexpressing the human familial APPswe and PS1 mutants (PS1/APP) with mice lacking the \( \beta_2 \)-AR gene (\( \beta_2 \)-KO). We found that the phosphorylation of tau at Ser-214, Ser-262, and Thr-181 was increased in the PFC of 6-month-old and 1-year-old PS1/APP mice compared with wild-type mice (Fig. 1, A–C, and data not shown). However, deletion of the \( \beta_2 \)-AR gene abolished the increases in phosphorylation of tau at Ser-
214 and Ser-262 and significantly reduced the increase in phosphorylation of tau at Thr-181 in the PFC of PS1/APP mice (Fig. 1, A–C, and data not shown). The phosphorylation of JNK1, GSK3α/β, and CaMK II was also increased in the PFC of 1-year-old PS1/APP animals, but the increases in phosphorylation of these proteins were greatly blunted in β2-KO/PS1/APP mice (Fig. 1, D–F).

We then applied primary PFC neurons isolated from wild-type and β2-KO animals to further dissect Aβ-induced β2AR signaling cascades in tau phosphorylation. Aβ (10⁻⁶ M) induced tau phosphorylation at Ser-214, Ser-262, and Thr-181 in wild-type PFC neurons, but the increases in tau phosphorylation were almost abolished at Ser-214 and Ser-262 and blunted significantly at Thr-181 in β2-KO neurons (Fig. 2, A–C). A minimal dose of 10⁻⁶ M of Aβ was effective to promote tau phosphorylation (data not shown). Meanwhile, a β2AR-selective antagonist, ICI118551, blocked Aβ-induced tau phosphorylation (Fig. 3, A–C). As a control, a general βAR antagonist, alprenolol (10⁻⁶ M), was effective to block Aα-induced (isoproterenol, 10⁻⁷ M) phosphorylation of tau at Ser-214 (A), Ser-262 (B), and Thr-181 (C) in primary WT PFC neurons were examined. The role of PKA in Aβ-induced phosphorylation of tau at Ser-214 (D), Ser-262 (E), and Thr-181 (F) in primary PFC neurons was examined. The roles of the adenylyl cyclase (AC) inhibitor (G), Epac inhibitor (H), and Epac activator (I) in Aβ-induced tau phosphorylation in primary PFC neurons were examined. Con, control group; Iso, isoproterenol; Alp, alprenolol; ICI, ICI118551; PKI, myristoylated PKA inhibitor.

**p < 0.01, versus control; &&, p < 0.01 versus isoproterenol-treated neurons; ##, p < 0.01 versus Aβ-treated neurons. n = 6.

**FIGURE 3. Role of β2AR signaling in Aβ-induced phosphorylation of tau.** The effects of a general βAR antagonist alprenolol (10⁻⁶ M) and a selective β2AR antagonist, ICI118551 (10⁻⁶ M) on Aβ-induced (10⁻⁶ M) phosphorylation of tau at Ser-214 (A), Ser-262 (B), and Thr-181 (C) in primary WT PFC neurons were examined. The role of PKA in Aβ-induced phosphorylation of tau at Ser-214 (D), Ser-262 (E), and Thr-181 (F) in primary PFC neurons was examined. The roles of the adenylyl cyclase (AC) inhibitor (G), Epac inhibitor (H), and Epac activator (I) in Aβ-induced tau phosphorylation in primary PFC neurons were examined. Con, control group; Iso, isoproterenol; Alp, alprenolol; ICI, ICI118551; PKI, myristoylated PKA inhibitor. **p < 0.01, versus control; &&, p < 0.01 versus isoproterenol-treated neurons; ##, p < 0.01 versus Aβ-treated neurons. n = 6.

214 and Ser-262 and significantly reduced the increase in phosphorylation of tau at Thr-181 in the PFC of PS1/APP mice (Fig. 1, A–C, and data not shown). The phosphorylation of JNK1, GSK3α/β, and CaMK II was also increased in the PFC of 1-year-old PS1/APP animals, but the increases in phosphorylation of these proteins were greatly blunted in β2-KO/PS1/APP mice (Fig. 1, D–F).

We then applied primary PFC neurons isolated from wild-type and β2-KO animals to further dissect Aβ-induced β2AR signaling cascades in tau phosphorylation. Aβ (10⁻⁶ M) induced tau phosphorylation at Ser-214, Ser-262, and Thr-181 in wild-type PFC neurons, but the increases in tau phosphorylation were almost abolished at Ser-214 and Ser-262 and blunted significantly at Thr-181 in β2-KO neurons (Fig. 2, A–C). A minimal dose of 10⁻⁶ M of Aβ was effective to promote tau phosphorylation (data not shown). Meanwhile, a β2AR-selective antagonist, ICI118551, blocked Aβ-induced tau phosphorylation (Fig. 3, A–C). As a control, a general βAR agonist, isoproterenol, also induced robust increases in tau phosphorylation, which was inhibited by a βAR antagonist, alprenolol (Fig. 3, A–C). Moreover, the effects of Aβ on tau phosphorylation at Ser-214 and Ser-262 were blocked by inhibition of adenylyl cyclase with 2,5-dideoxyadenosine-3-triphosphate tetrasodium (10⁻⁴ M, Fig. 3, G) or inhibition of PKA inhibitor with membrane-permeable myristoylated PKI (10⁻⁵ M, D and E). The Aβ-induced tau phosphorylation was not affected by the Epac inhibitor brefeldin A (10⁻⁷ M), and treating the cells with the Epac-selective activator 8-CPT-2Me-cAMP (10⁻⁷ M) for 5 min did not induce tau phosphorylation (Fig. 3, H and I). These results indicate that tau phosphorylation at Ser-214 and Ser-262 is...
primarily dependent on β2-AR-adenyl cyclase-PKA signaling. In comparison, Aβ-induced tau phosphorylation at Thr-181 was only blocked partially by inhibition of PKA with PKI (10⁻⁵ M, Fig. 3F).

We then attempted to define intracellular signaling cascades involved in Aβ-induced phosphorylation of tau via β₂-AR activation. A JNK inhibitor, SP600125 (2 × 10⁻⁷ M, IC₅₀ = 4–9 × 10⁻⁸ M), blocked the phosphorylation at Thr-181 and significantly blunted the phosphorylation of tau at Ser-214 and Ser-262 (Fig. 4D–F). Moreover, Aβ-induced (10⁻⁶ M) phosphorylation of JNK was partially inhibited by PKI (10⁻⁵ M) but not by the Epac inhibitor brefeldin A (10⁻⁷ M, Fig. 4E). These data suggest that a β₂-AR-PKA-JNK pathway contributes to the Aβ-induced tau phosphorylation. In comparison, acute Aβ treatment for 5 min induced minimal change in the phosphorylation of CaMK II in either wild-type or β₂-KO neurons (Fig. 4F and G), indicating that the increase in the phosphorylation of CaMK II observed in vivo, shown in Fig. 1, likely requires additional

**FIGURE 4. Roles of JNK, GSK3β, CaMK II, and PKC in Aβ-induced phosphorylation of tau in primary PFC neurons.** The effects of protein kinase inhibitors on Aβ-induced phosphorylation of tau at Ser-214 (A), Ser-262 (B), and Thr-181 (C) were investigated. The Aβ-induced phosphorylation of JNK at Thr-183/185 (D) and CaMK II at Thr-286 (F) in primary PFC neurons of WT and β₂-KO was examined. The effects of a PKA inhibitor, myristoylated PKI, and an Epac inhibitor, brefeldin A (BFA), on Aβ-induced phosphorylation of JNK at Thr-183/185 (E) and CaMK II at Thr-286 (G) were investigated. Con, control group; SP600125, JNK inhibitor; BIO, GSK3β inhibitor; KN-93, CaMK II inhibitor; Calphostin C, PKC inhibitor. *, p < 0.05; **, p < 0.01 versus control; #, p < 0.05 versus Aβ-treated neurons. n = 6.
These data essentially rule out a role of arrestins in Aβ/H11011/67 of ERK1/2 (Fig. 5A). However, Aβ upstream enzyme of ERK1/2, with U0126 (2μM) phosphorylation of tau. However, inhibition of MEK, an Aβ pathway.

Activation of other receptors and ion channels under chronic conditions.

Activation of β2AR also induces MAPK signaling via arrestins. Here, Aβ (10^{-6} μM) induced a β2AR-dependent activation of ERK1/2 (Fig. 5A). However, Aβ failed to increase phosphorylation of ERK1/2 in arrestin-3 KO neurons, indicating that arrestin-3 is required for Aβ-induced activation of ERK1/2 (Fig. 5, B and C). As a control, the βAR agonist isoproterenol (10^{-7} μM) increased the phosphorylation of ERK1/2 in both arrestin-2- and arrestin-3-KO neurons (Fig. 5, B and C). Because MAP kinases can also promote phosphorylation of tau, we tested the role of the arrestin-ERK1/2 pathway in Aβ-induced phosphorylation of tau in PFC neurons. In PFC neurons lacking arrestin-2 and/or arrestin-3, Aβ (10^{-6} μM) increased the phosphorylation of tau at Ser-214, Ser-262, and Thr-181 (Fig. 6, A–C and E). These data essentially rule out a role of arrestins in Aβ-induced phosphorylation of tau. However, inhibition of MEK, an upstream enzyme of ERK1/2, with U0126 (2 × 10^{-7} μM, IC_{50} = 6–7 × 10^{-8} μM) partially blocked the phosphorylation of tau at Thr-181, but not at Ser-214 and Ser-262 (Fig. 6D). Together, these data indicate that Aβ induces activation of MEK, which phosphorylates tau at Thr-181 and ERK1/2 through different signaling machineries, and only the ERK1/2 phosphorylation is dependent on arrestin-3 (Fig. 7).

In agreement with published literature, we found that the density of dendritic spines in the deep layer of the PFC in 1-year-old and 6-month-old PS1/APP mice was decreased. However, deletion of the β2AR gene reversed the decrease (Fig. 8, A–C). Unlike the relatively even distribution of synapses in the deep layer of the PFC in WT mice, PS1/APP mice displayed regions with a dramatically decreased number of synapses, as indicated by synapsin I staining (Fig. 8, D and E), and surrounding synapses remained in clusters (arrows). Deletion of the β2AR gene in PS1/APP mice yielded a distribution of synapsin I positively stained synapses similar to those in WT or β2-KO mice (Fig. 8, D and E). To assess the cognitive role of β2AR in PS1/APP transgenic AD animals, we tested learning and memory in 1-year-old mice in a novel object recognition paradigm. We found that PS1/APP mice showed impaired learning and memory, whereas β2-KO/PS1/APP mice performed significantly better than PS1/APP mice (Fig. 9A). Knockout of the β2AR gene itself tended to improve learning and memory in 1-year-old mice (Fig. 9A). However, it tended to impair learning and memory in 6-month-old mice (Fig. 9D). In the training
session, one-year-old animals in each group showed a similar preference for the reference object (Fig. 9B). The total exploration time in PS1/APP and β2-KO/PS1/APP animals in the training session was similar, indicating similar locomotor activity in these mice (Fig. 9C).

**DISCUSSION**

Recent epidemiological studies suggest that β blockers may reduce the incidence of AD in patients suffering from hypertension and are associated with delay of functional decline in sporadic AD patients (13). Among three subtypes in the βAR family, both β1AR and β2AR play important roles in cognition and stress-dependent behaviors (17, 18). Accumulating evidence suggests that β1AR and β2AR, especially β2AR, may be involved in AD pathogenesis through effects on Aβ production or inflammation (14, 19, 29) and that polymorphisms of β2AR contribute to sporadic late-onset AD, which may be related to the availability and response of β2AR (13, 16). Our previous studies have shown that Aβ can bind to β2AR and induce allosteric activation of the receptor that leads to cAMP/PKA- and GRK/arrestin-mediated cell signaling (19, 22, 23). In this study,

**FIGURE 7.** The β2AR-signaling machinery regulates Aβ-induced tau phosphorylation. Aβ, amyloid β peptide; β2, β2 receptor; Arr3, arrestin 3.

**FIGURE 8.** Deletion of the β2AR gene reduces loss of dendritic spines and synapses in the brain of senile mice. A, representative figures show the density of dendritic spines in the deep layer of the PFC of 1-year-old wild-type, β2-KO, PS1/APP, and β2-KO/PS1/APP mice. B, semiquantitative analysis of the density of dendritic spines in A (n = 80). C, representative figures show the density of dendritic spines in deep layer of PFC of 6-month-old WT, β2-KO, PS1/APP, and β2-KO/PS1/APP mice. Synapsin-I staining for synapses in the deep layer of the PFC of 1-year-old WT, β2-KO, PS1/APP, and β2-KO/PS1/APP mice. Synapsin-I positively stained clusters of synapses surrounded by blank areas are indicated with arrows. E, semiquantification of the density of synapses in D. *, p < 0.05; **, p < 0.01 versus WT; ##, p < 0.01 versus PS1/APP mice. n = 8—15.
we find that $\beta_2$AR plays a necessary role in $\Aβ$-induced tau phosphorylation at Ser-214, Ser-262, and Thr-181 in vivo. Deletion of the $\beta_2$AR gene prevents tau hyperphosphorylation, loss of dendritic spines and synapses, and impairment of learning and memory in a transgenic AD animal model. This study places $\beta_2$AR as an essential link between increasing $\Aβ$ and tau phosphorylation levels in the brain, which are both hallmarks of AD pathogenesis.

In tauopathies such as AD, frontotemporal dementia, and Parkinson disease, tau is hyperphosphorylated abnormally at multiple serine/threonine sites. In this study, one-year-old PS1/APP transgenic AD animals show hyperphosphorylation of tau at Ser-214, Ser-262, and Thr-181. Deletion of the $\beta_2$AR gene significantly attenuates the phosphorylation of tau at Thr-181 and completely blocks the phosphorylation of tau at Ser-214 and Ser-262 in vivo and in vitro, suggesting that $\beta_2$AR is a primary receptor for $\Aβ$-induced phosphorylation of tau at these sites. PKA and CaMK II are downstream from $\beta_2$AR. Previous studies have shown that both PKA and CaMK II readily phosphorylate tau. However, PKA phosphorylates tau to a significantly greater extent with a broader range of the sites than CaMK II (30, 31). Phosphorylation of tau by PKA also significantly decreases tubulin binding (30). In a tandem mass spectrometry study, CaMK II phosphorylated recombinant human tau at the sites, including Ser-214 and Ser-262, that may produce paired helical filament tau (32). Here, we find that $\Aβ$-induced phosphorylation of tau at Ser-214 and Ser-262 is primarily dependent on PKA, whereas the phosphorylation at Thr-181 is partially inhibited by PKA inhibitor PKI. These data support that $\beta_2$AR signals through PKA in $\Aβ$-induced tau phosphorylation. In comparison, inhibition of CaMK II does not block the $\Aβ$-induced phosphorylation at these sites in PFC neurons. It has been shown that $\Aβ$ may induce hyperactivities in AMPA receptors under electric stimulation in PFC slices (22). Here, acute treatment with $\Aβ$ alone for 5 min without electric stimulation does not induce significant phosphorylation of CaMK II in both wild-type and $\beta_2$KO PFC neurons, probably because of lack of glutamate released from presynapses for activation of AMPA receptors. Nevertheless, one-year-old PS1/APP transgenic animals show an increased phosphorylation of CaMK II that is dependent on expression of $\beta_2$AR. Thus, a possible role of CaMK II for $\Aβ$-induced and $\beta_2$AR-mediated tau phosphorylation in vivo remains to be addressed.

In addition, the JNK pathway amplifies and drives subcellular changes in tau phosphorylation (1) and plays key role in tau phosphorylation in AD models (33). GSK3$β$ is a major physiological tau kinase that requires priming phosphorylation at Ser-404 to further phosphorylate tau at paired helical filament 1 (34). In isolated PFC neurons, a JNK inhibitor totally blocks the phosphorylation of tau at Thr-181 and significantly attenuates the phosphorylation of Tau at Ser-214 and Ser-262 induced by $\Aβ$. Although $\Aβ$ induces phosphorylation of JNK in isolated PFC neurons via a $\beta_2$AR-PKA pathway, the phosphorylation of JNK is only partially blunted in the PFC of 1-year-old $\beta_2$AR-KO/PS1/APP animals. These data indicate that $\beta_2$AR is a major receptor associated with JNK phosphorylation and that other $\Aβ$-induced receptor pathways or $\Aβ$ deposition-induced inflammation can also promote JNK phosphorylation in vivo. Together, $\Aβ$ induces JNK phosphorylation through activating $\beta_2$AR-PKA signaling and other signaling mechanisms in which
PKA and JNK independently contribute to tau phosphorylation at Ser-214, Ser-262, and Thr-181. We also find that Aβ can act as a β2AR-arrestin-MAPK pathway in PFC neurons. Surprisingly, we find that MEK, but not downstream ERK1/2 in the MAPK pathway, contributes to phosphorylation of tau at Thr-181. MEK-mediated tau phosphorylation does not require expression of arrestin-2 or arrestin-3, the non-visual arrestins function downstream of β2AR in the brain. In comparison, arrestin-3 is required for the Aβ-induced ERK1/2 phosphorylation. These data indicate that Aβ-induced MEK phosphorylation leads to two divergent pathways: an arrestin-3-dependent ERK1/2 activation and an arrestin-independent tau phosphorylation.

Genetic data have implied that deranged tau-microtubule interactions induced either by phosphorylation or increased levels of tau, contribute to or even are sufficient to cause synaptic and dendritic degeneration in primary tauopathies (35–37). It has been reported that transfection of tau in mature neurons leads to an improper distribution of tau into the somatodendritic compartment with concomitant degeneration of synapses, as seen by the disappearance of spines and presynaptic and postsynaptic markers (3, 7). In this study, there is a degeneration of synapses shown by synapsin I staining and dendritic spines in the deep layer of the PFC of PS1/APP double-transgenic animals. Deletion of the β2AR gene in PS1/APP animals reverses the degenerative effects. These findings argue for a potential beneficial role of inhibition of β2AR in altering the pathological course of AD. Conclusive evidence comes from the behavioral experiments. In the novel object recognition test, learning and memory deficits are present in 1-year-old PS1/APP animals. Deletion of the β2AR gene rescues the outcome resulting from overexpressing mutant PS1 and APP genes from human familial AD. It is worth noting that 1-year-old β2-KO animals show a tendency to perform better than wild-type animals in the behavioral test. However, 6-month-old β2-KO animals show a tendency to have slightly decreased learning and memory. In either case, deletion of the β2AR gene in PS1/APP animals has beneficial effects in the test. Taken together, the cellular and behavioral experiments in this study provide evidence that β2AR may represent a potential therapeutic target for preventing the development of AD.

REFERENCES


