**β**<sub>2</sub> Adrenergic Receptor, Protein Kinase A (PKA) and c-Jun N-terminal Kinase (JNK) Signaling Pathways Mediate Tau Pathology in Alzheimer Disease Models**

Received for publication, August 31, 2012, and in revised form, February 18, 2013. Published, JBC Papers in Press, February 19, 2013, DOI 10.1074/jbc.M112.415141

Dayong Wang<sup>1</sup>, Qin Fu<sup>1,5</sup>, Yuan Zhou<sup>1</sup>, Bing Xu<sup>1</sup>, Qian Shi<sup>5</sup>, Benedict Igwe<sup>‡</sup>, Lucas Matt<sup>¶</sup>, Johannes W. Hell<sup>§</sup>, Elena V. Wisely<sup>1</sup>, Salvatore Oddo<sup>1</sup>, and Yang K. Xiang<sup>‡,§</sup>

From the<sup>1</sup>Department of Molecular and Integrative Physiology, School of Molecular and Cellular Biology, University of Illinois at Urbana-Champaign, Urbana, Illinois 61801, the<sup>2</sup>Department of Pharmacology, University of California at Davis, Davis, California 95616, the<sup>3</sup>Department of Pharmacology, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, Hubei 430030, China, and the<sup>4</sup>Department of Physiology, University of Texas Health Science Center, San Antonio, Texas 78229

**Background:** Accumulating evidence indicates that β receptors (βAR) 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 protein. In AD, we identify β<sub>2</sub>-adrenergic receptor (β<sub>2</sub>AR) 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 and Thr-181 is increased significantly. Deletion of the β<sub>2</sub>AR 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 β<sub>2</sub>AR 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)<sup>2</sup>, 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 β<sub>2</sub>AR may be involved in AD pathogenesis through effects on Aβ production and inflammation (14, 15). Another study has shown recently that polymorphism in β<sub>2</sub>AR contributes to sporadic late-onset AD, which may be related to the availability and response of β<sub>2</sub>AR (13, 16). Meanwhile, β<sub>2</sub>AR 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

---

*This work was supported by a new investigator research grant from the Alzheimer’s Association (to Y.K.X.) and by The National Alliance for Research on Schizophrenia and Depression (NARSAD) Young Investigator Award and Alzheimer’s Disease Research Fund from the Illinois Department of Public Health (to D.W.).

<sup>1</sup>To whom correspondence should be addressed: Department of Pharmacology, University of California at Davis, Davis, CA 95616. E-mail: ykxiang@ucdavis.edu.

<sup>2</sup>The abbreviations used are: AD, Alzheimer disease; Aβ, amyloid β peptide; β<sub>2</sub>AR, β<sub>2</sub>-adrenergic receptor; PKA, protein kinase A; E<sub>pac</sub>, exchange protein activated by cAMP; APP, amyloid precursor protein; PFC, prefrontal cortex.
kinase/arrin-dependent signal transduction, which is pre-
synaptic activity-independent and requires the N terminus of
β2AR (19–22). Although a prolonged treatment with Aβ
induces GRK/arrin 3-dependent internalization and degra-
dation 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 arrin signaling can trigger the
phosphorylation of MAPK and JNK that may phosphorylate tau
(24), and the activation of the exchange protein activated by
phosphorylation of MAPK and JNK that may phosphorylate tau
internalization-associated arrin signaling can trigger the
activity are partially preserved, reaching a balance between
neurotransmission, the intracellular levels of cAMP and PKA

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-trans-
genic (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 Lon-
don (V717I) familial AD mutations and human presenilin 1
gene harboring two-familial AD mutations, M146L and L286V
(26). PS1/APP mice were cross-bred with β2-KO mice to pro-
duce 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 × 105–
106 cells/ml in DMEM/F12 medium (1:1) containing 10% FBS,
1% insulin-transferrin-selenium supplement, 25 ng/ml nerve
growth factor, 1 mM glutamine, 20 mM water-soluble progester-
one, and 100 mM 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 (Biopep-
tide, 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 mono-
mers, 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 Scien-
tific, MD) was used to stain dendritic spines of neurons in the
depth 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 micro-
scope equipped with DIC objective lenses. All spines observable
along 100-μm dendritic segments at least 25 μm from the cell
soma were counted.

Immunofluorescence Microscopy—Wild-type, β2-KO, PS1/
APP, and β2-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 dehydra-
tion in sucrose, the brains were frozen in Tissue-Tek O.C.T
compound (VWR LabShop, IL), and slices were cut at a thick-
ess 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 per-
formed 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 phos-
phorylated tau of both human and mouse. After washing, mem-
branes 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 ses-
sions: habituation, training, and retention sessions. Each mouse
was habituated individually to the box with 10 min of explora-
tion 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 explor-
ing the two objects was recorded for 10 min by the experi-
menter with two stopwatches. Exploration of an object was
counted if the mouse moved away from the box for more than 2 cm
or touched it with the nose. During the reten-
tion 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 β2AR signaling in tau pathology in relationship with Aβ in AD, we cross-bred the AD animal model overexpressing the human familial APPswe and PS1 mutants (PS1/APP) with mice lacking the β2AR gene (β2-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 β2AR gene abolished the increases in phosphorylation of tau at Ser-

**FIGURE 1. Deletion of the β2AR gene ameliorates hyperphosphorylation of tau in AD animal brain.** Phosphorylation of tau at Ser-214 (A), Ser-262 (B), Thr-181 (C), JNK (D), GSK3α/β (E), and CaMK II (F) in the PFC tissues of 1-year-old WT, β2-KO, PS1/APP, and β2-KO/PS1/APP mice. ***, p < 0.01** versus WT mice; #, *p* < 0.05 versus PS1/APP mice; **##**, *p* < 0.01 versus PS1/APP mice. *n* = 6.

**FIGURE 2. Time course of Aβ-induced phosphorylation of tau in WT and β2-KO PFC neurons.** Aβ-induced phosphorylation of tau at Ser-214 (A), Ser-262 (B), and Thr-181 (C) in primary PFC neurons of WT and β2-KO mice was examined. ***, p < 0.01** between WT and β2-KO by two-way analysis of variance. *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, GSK3β/H9251, 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 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 β2-selective antagonist, ICI118551, blocked Aβ-induced tau phosphorylation (Fig. 3, A–C). As a control, a general βAR antagonist, alprenolol (10^{-6} M), efficiently blocked Aβ-induced 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 PKI 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^{-5} 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.
primarily dependent on $\beta_2$AR-adenyl cyclase-PKA signaling. In comparison, $A\beta$-induced tau phosphorylation at Thr-181 was only blocked partially by inhibition of PKA with PKI (10^{-5} M, Fig. 3).

We then attempted to define intracellular signaling cascades involved in $A\beta$-induced phosphorylation of tau via $\beta_2$AR activation. A JNK inhibitor, SP600125 (2 $\times$ 10^{-7} M, IC$_{50} = 4$ $\times$ 9 $\times$ 10^{-8} M), blocked the phosphorylation at Thr-181 and significantly blunted the phosphorylation of tau at Ser-214 and Ser-262 (Fig. 4, A–C). In comparison, $A\beta$-induced tau phosphorylation at Thr-181 was only blocked partially by inhibition of PKA with PKI (10^{-5} M, Fig. 3F).

FIGURE 4. Roles of JNK, GSK3$, \beta$, CaMK II, and PKC in $A\beta$-induced phosphorylation of tau in primary PFC neurons. The effects of protein kinase inhibitors on $A\beta$-induced phosphorylation of tau at Ser-214 (A), Ser-262 (B), and Thr-181 (C) were investigated. The $A\beta$-induced phosphorylation of JNK at Thr-183/185 (D) and CaMK II at Thr-286 (F) in primary PFC neurons of WT and $\beta_2$-KO was examined. The effects of a PKA inhibitor, myristoylated PKI, and an Epac inhibitor, brefeldin A (BFA), on $A\beta$-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, GSK3$\beta$ inhibitor; KN-93, CaMK II inhibitor; Calphostin C, PKC inhibitor. *, $p < 0.05$; **, $p < 0.01$ versus control; #, $p < 0.05$ versus $A\beta$-treated neurons. n = 6.
These data essentially rule out a role of arrestins in Aβ. However, Aβ-induced (10⁻⁶ M) phosphorylation of ERK1/2 in primary PFC neurons isolated from WT and β₂-KO mice was investigated. Aβ-induced ERK1/2 phosphorylation in primary Arr-2-KO (B) and Arr-3-KO (C) PFC neurons was examined. Iso, isoproterenol; Arr, arrestin. *, p < 0.05; **, p < 0.01 versus basal level. n = 6.

activation of other receptors and ion channels under chronic conditions.

Activation of β₂AR also induces MAPK signaling via arrestins. Here, Aβ (10⁻⁶ M) 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 M) 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 M) 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 M, IC₅₀ = 6–7 × 10⁻⁸ M) 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 $\beta_2$-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 $\beta$ 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 $\beta$AR family, both $\beta_1$AR and $\beta_2$AR play important roles in cognition and stress-dependent behaviors (17, 18). Accumulating evidence suggests that $\beta_1$AR and $\beta_2$AR, especially $\beta_2$AR, may be involved in AD pathogenesis through effects on Aβ production or inflammation (14, 19, 29) and that polymorphisms of $\beta_2$AR contribute to sporadic late-onset AD, which may be related to the availability and response of $\beta_2$AR (13, 16). Our previous studies have shown that Aβ can bind to $\beta_2$AR 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 $\beta_2$AR-signaling machinery regulates Aβ-induced tau phosphorylation. Aβ, amyloid $\beta$ peptide; $\beta_2$, $\beta_2$ receptor; Arr3, arrestin 3.

**FIGURE 8.** Deletion of the $\beta_2$AR 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, $\beta_2$-KO, PS1/APP, and $\beta_2$-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, $\beta_2$-KO, PS1/APP, and $\beta_2$-KO/PS1/APP mice. D, synapsin I staining for synapses in the deep layer of the PFC of 1-year-old WT, $\beta_2$-KO, PS1/APP, and $\beta_2$-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 β2AR plays a necessary role in Aβ-induced tau phosphorylation at Ser-214, Ser-262, and Thr-181 in vivo. Deletion of the β2AR 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 β2AR 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 in vitro and in vivo. Deletion of the β2AR 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 β2AR is a primary receptor for Aβ-induced phosphorylation of tau at these sites. PKA and CaMK II are downstream from β2AR. 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 β2AR 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 β2KO 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 β2AR. Thus, a possible role of CaMK II for Aβ-induced and β2AR-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). GSK3β 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 β2AR-PKA pathway, the phosphorylation of JNK is only partially blunted in the PFC of 1-year-old β2AR-KO/PS1/APP animals. These data indicate that β2AR 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 β2AR-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 activate 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 transferase 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 β2KO animals show a tendency to perform better than wild-type animals in the behavioral test. However, 6-month-old β2KO 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

1. Vogel, J., Anand, V. S., Ludwig, B., Nawoschik, S., Dunlop, J., and Braithwaite, S. P. (2009) The JNK pathway amplifies and drives subcellular changes in tau phosphorylation. Neuropharmacology 57, 539–550

2. Hall, G. F., Chu, B., Lee, G., and Yao, J. (2000) Human tau filaments induce microtubule and synapse loss in an in vivo model of neurofilibrillary degenerative disease. J. Cell Sci. 113, 1373–1387

3. Thies, E., and Mandellkow, E. M. (2007) Missorting of tau in neurons causes degeneration of synapses that can be rescued by the kinase MARK2/Par-1. J. Neurosci. 27, 2896–2907

4. Mattson, M. P. (2004) Pathways towards and away from Alzheimer’s disease. Nature 430, 631–639

5. Giumfrida, M. L., Caraci, F., Pignataro, B., Cataldo, S., De Bona, P., Bruno, V., Molinari, G., Pappalardo, G., Messina, A., Palmigiano, A., Garozzo, D., Nicoletti, F., Rizzarelli, E., and Copani, A. (2009) β-Amyloid monomers are neuroprotective. J. Neurosci. 29, 10582–10587

6. Ma, Q. L., Yang, F., Rosario, E. R., Ubeda, O. J., Beech, W., Gant, D. I., Chen, P. P., Hudspeth, B., Chen, C., Zhao, Y., Vinters, H. V., Frautschy, S. A., and Cole, G. M. (2009) β-Amyloid oligomers induce phosphorylation of tau and inactivation of insulin receptor substrate via c-Jun N-terminal kinase signaling. Suppression by omega-3 fatty acids and curcumin. J. Neurosci. 29, 9078–9089

7. Zempel, H., Thies, E., Mandellkow, E., and Mandellkow, E. M. (2010) Aβ oligomers cause localized Ca(2+) elevation, missorting of endogenous tau into dendrites, tau phosphorylation, and destruction of microtubules and spines. J. Neurosci. 30, 11938–11950

8. Mitsuyama, F., Futatsugi, Y., Okuya, M., Karagiozov, K., Peev, N., Kato, Y., Kanno, T., Sano, H., and Koide, T. (2009) Amyloid β. A putative intraspinal microtubule-depolymerizer to induce synapse-loss or dendritic spine shortening in Alzheimer’s disease. Ital. J. Anat. Embryol. 114, 109–120

9. Mitsuyama, F., Futatsugi, Y., Okuya, M., Kawase, T., Karagiozov, K., Kato, Y., Kanno, T., Sano, H., Nagao, S., and Koide, T. (2012) Stimulation-dependent intraspinal microtubule and synaptic failure in Alzheimer’s disease mice. PLoS ONE 5, e15477

10. Bittner, T., Fuhrmann, M., Burgold, S., Ochs, S. M., Hoffmann, N., Mittereiger, G., Kretzschmar, H., LaFerla, F. M., and Herms, J. (2010) Multiple events lead to dendritic spine loss in triple transgenic Alzheimer’s disease mice. J. Biol. Chem. 288, 2949–2961

11. Hoover, B. R., Reed, M. N., Su, J., Penrod, R. D., Kotilinek, L. A., Grant, M. K., Pitsick, R., Carlson, G. A., Lanier, L. M., Yuan, L. L., Ashe, K. H., and Liao, D. (2010) Tau mislocalization to dendritic spines mediates synaptic dysfunction independently of neurodegeneration. Neurodegener. 68, 1067–1081

12. Violin, J. D., DiPilato, L. M., Yildirim, N., Elston, T. C., Zhang, J., and Lefkowitz, R. J. (2008) β2-adrenergic receptor signaling and desensitization elucidated by quantitative modeling of real time cAMP dynamics. J. Biol. Chem. 283, 2949–2961

13. Rosenberg, P. B., Mielke, M. M., Tschanz, J., Cook, L., Corcoran, C., Hayden, K. M., Norton, M., Rabins, P. V., Green, R. C., Welsh-Bohmer, K. A., Breitner, J. C., Munger, R., and Lyketsos, C. G. (2008) Effects of cardiovascular medications on rate of functional decline in Alzheimer disease. Am. J. Geriatr. Psychiatry 16, 883–892

14. Yi, Y., Zhao, X., Bao, G., Zou, L., Teng, L., Wang, Z., Song, M., Xiong, J., Bai, Y., and Pei, G. (2006) Activation of β2-adrenergic receptor stimulates γ-secretase activity and accelerates amyloid plaque formation. Nat. Med. 12, 1390–1396

15. Yu, J. T., Wang, N. D., Ma, T., Jiang, H., Guan, J., and Tan, L. (2011) Roles of β2-adrenergic receptors in Alzheimer’s disease. Implications for novel therapeutics. Brain Res. Bull. 84, 111–117

16. Yu, J. T., Tan, L., Ou, J. R., Zhu, J. X., Liu, K., Song, J. H., and Sun, Y. P. (2008) Polymorphisms at the β2-adrenergic receptor gene influence Alzheimer’s disease’s susceptibility. Brain Res. 1210, 216–222

17. Morilak, D. A., Barrera, G., cheverria, D. J., Garcia, A. S., Hernandez, A., Ma, S., and Petre, C. O. (2005) Role of brain norepinephrine in the behavioral response to stress. Prog. Neuropsychopharmacol. Biol. Psychiatry 29, 1214–1224

18. Ramos, B. P., and Arnsten, A. F. (2007) Adrenergic pharmacology and cognition. Focus on the prefrontal cortex. Pharmacol. Ther. 113, 523–536

19. Igbaivo, U., Johnson-Anuna, L. N., Rossello, X., Butterick, T. A., Sun, G. Y., and Wood, W. G. (2006) Amyloid β-protein 1–42 increases cAMP and apolipoprotein E levels which are inhibited by β1 and β2-adrenergic receptor antagonists in mouse primary astrocytes. Neuroscience 142, 655–660

20. Prapong, T., Uemura, E., and Hsu, W. H. (2001) G protein and cAMP-dependent protein kinase mediate amyloid β-peptide inhibition of neuronal glucose uptake. Exp. Neurol. 167, 59–64

21. Cheverria, V., Ducatenzeiler, A., Chen, C. H., and Cuello, A. C. (2005) Endogenous β-amyloid peptide synthesis modulates cAMP response element-regulated gene expression in PC12 cells. Neuroscience 135, 1193–1202

22. Wang, D., Govindaiith, G., Liu, R., De Arcangelis, V., Cox, C. L., and Xiang, Y. K. (2010) Binding of amyloid β peptide to β2 adrenergic receptor induces PKA-dependent AMPA receptor hyperactivity. FASEB J. 24, 3511–3521

23. Wang, D., Yuen, E. Y., Zhou, Y., Yan, Z., and Xiang, Y. K. (2011) Amyloid β peptide(1–42) induces internalization and degradation of β2 adrenergic receptors in prefrontal cortical neurons. J. Biol. Chem. 286, 10306–10317
24. Guo, C., and Whitmarsh, A. J. (2008) The β-arrestin-2 scaffold protein promotes c-Jun N-terminal kinase-3 activation by binding to its nonconserved N terminus. *J. Biol. Chem.* 283, 15093–15091

25. Hochbaum, D., Tanos, T., Ribeiro-Neto, F., Altschuler, D., and Coso, O. A. (2003) Activation of JNK by Epac is independent of its activity as a Rap guanine nucleotide exchanger. *J. Biol. Chem.* 278, 33738–33746

26. Oakley, H., Cole, S. L., Logan, S., Maus, E., Shao, P., Craft, J., Guillozet-Bongaarts, A., Ohno, M., Disterhoft, J., Van Eldik, L., Berry, R., and Vassar, R. (2006) Intraneuronal β-amyloid aggregates, neurodegeneration, and neuron loss in transgenic mice with five familial Alzheimer’s disease mutations. Potential factors in amyloid plaque formation. *J. Neurosci.* 26, 10129–10140

27. Nagai, T., Yamada, K., Kim, H. C., Kim, Y. S., Noda, A., Nabeshima, Y., and Nabeshima, T. (2003) Cognition impairment in the genetic model of aging klotho gene mutant mice. A role of oxidative stress. *FASEB J.* 17, 50–52

28. Wang, D., Noda, Y., Zhou, Y., Mouri, A., Mizoguchi, H., Nitta, A., Chen, W., and Nabeshima, T. (2007) The allosteric potentiation of nicotinic acetylcholine receptors by galantamine ameliorates the cognitive dysfunc- tion in β amyloid25–35 i.c.v.-injected mice. Involvement of dopaminergic systems. *Neuropsychopharmacology* 32, 1261–1271

29. Johnson, J. D., Cortez, V., Kennedy, S. L., Foley, T. E., Hanson, H., 3rd, and Fleshner, M. (2008) Role of central β-adrenergic receptors in regulating proinflammatory cytokine responses to a peripheral bacterial challenge. *Brain Behav. Immun.* 22, 1078–1086

30. Johnson, G. V. (1992) Differential phosphorylation of tau by cyclic AMP-dependent protein kinase and Ca²⁺/calmodulin-dependent protein kinase II. Metabolic and functional consequences. *J. Neurochem.* 59, 2056–2062

31. Litersky, J. M., Johnson, G. V., Jakes, R., Goedert, M., Lee, M., and Seubert, P. (1996) Tau protein is phosphorylated by cyclic AMP-dependent protein kinase and calcium/calmodulin-dependent protein kinase II within its microtubule-binding domains at Ser-262 and Ser-356. *Biochem. J.* 316, 655–660

32. Yoshimura, Y., Ichinose, T., and Yamauchi, T. (2003) Phosphorylation of tau protein to sites found in Alzheimer’s disease brain is catalyzed by Ca²⁺/calmodulin-dependent protein kinase II as demonstrated tandem mass spectrometry. *Neurosci. Lett.* 353, 185–188

33. Ploia, C., Antoniou, X., Sclip, A., Grande, V., Cardinetti, D., Colombo, A., Canu, N., Benussi, L., Ghidoni, R., Forloni, G., and Borsello, T. (2011) JNK plays a key role in tau hyperphosphorylation in Alzheimer’s disease models. *J. Alzheimers Dis.* 26, 315–329

34. Sato, S., Tatebayashi, Y., Akagi, T., Chui, D. H., Murayama, M., Miyasaka, T., Planel, E., Tanemura, K., Sun, X., Hashikawa, T., Yoshioka, K., Ishiguro, K., and Takashima, A. (2002) Aberrant tau phosphorylation by glycogen synthase kinase-3β and JNK3 induces oligomeric tau fibrils in COS-7 cells. *J. Biol. Chem.* 277, 42060–42065

35. Jaworski, T., Kugler, S., and Van Leuven, F. (2010) Modeling of tau-mediated synaptic and neuronal degeneration in Alzheimer’s disease. *Int. J. Alzheimers Dis.* 2010, 1–10

36. Jaworski, T., Lechat, B., Demedts, D., Giels, L., Devijver, H., Borghgraef, P., Duimel, H., Verheyen, F., Kügler, S., and Van Leuven, F. (2011) Dendritic degeneration, neurovascular defects, and inflammation precede neuronal loss in a mouse model for tau-mediated neurodegeneration. *Am. J. Pathol.* 179, 2001–2015

37. Jaworski, T., Dewachter, I., Lechat, B., Croes, S., Tormont, A., Demedts, D., Borghgraef, P., Devijver, H., Filipkowski, R. K., Kaczmarek, L., Kügler, S., and Van Leuven, F. (2009) AAV-tau mediates pyramidal neurodegeneration by cell-cycle re-entry without neurofibrillary tangle formation in wild-type mice. *PLoS ONE* 4, e7280