

ALZHEIMER'S DISEASE

 β -amyloid redirects norepinephrine signaling to activate the pathogenic GSK3 β /tau cascadeFang Zhang^{1*}, Mary Gannon^{1*}, Yunjia Chen¹, Shun Yan², Sixue Zhang³, Wendy Feng¹, Jiahui Tao¹, Bingdong Sha¹, Zhenghui Liu⁴, Takashi Saito^{5†}, Takaomi Saido⁵, C. Dirk Keene⁶, Kai Jiao², Erik D. Roberson⁷, Huaxi Xu⁸, Qin Wang^{1‡}Copyright © 2020
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The brain noradrenergic system is critical for normal cognition and is affected at early stages in Alzheimer's disease (AD). Here, we reveal a previously unappreciated direct role of norepinephrine signaling in connecting β -amyloid (A β) and tau, two key pathological components of AD pathogenesis. Our results show that A β oligomers bind to an allosteric site on α_{2A} adrenergic receptor (α_{2A} AR) to redirect norepinephrine-elicited signaling to glycogen synthase kinase 3 β (GSK3 β) activation and tau hyperphosphorylation. This norepinephrine-dependent mechanism sensitizes pathological GSK3 β /tau activation in response to nanomolar accumulations of extracellular A β , which is 50- to 100-fold lower than the amount required to activate GSK3 β by A β alone. The significance of our findings is supported by *in vivo* evidence in two mouse models, human tissue sample analysis, and longitudinal clinical data. Our study provides translational insights into mechanisms underlying A β proteotoxicity, which might have strong implications for the interpretation of A β clearance trial results and future drug design and for understanding the selective vulnerability of noradrenergic neurons in AD.

INTRODUCTION

Alzheimer's disease (AD) and related dementia affect nearly 50 million people globally, and there is currently no effective therapy to cure this devastating disease or to slow its progression. Strong genetic and experimental evidence indicates toxic β -amyloid (A β) peptides as a key driving factor of AD pathogenesis (1–4). However, the failure of multiple clinical trials that directly target A β in the brain suggests that simply reducing A β burden does not necessarily result in alleviation of cognitive impairment (5). The microtubule-associated protein tau is an essential mediator of A β toxicity (6, 7). Hyperphosphorylated and aggregated tau disrupts neuronal functions and plasticity, and spreading of tau pathology positively correlates with cognitive impairment in AD (8–10). Yet, the molecular pathway from A β to tau pathology remains elusive, presenting a major gap in in-depth understanding of the pathological cascade of AD.

Brain locus coeruleus (LC) noradrenergic neurons are highly vulnerable in AD and degenerate at early stages of the disease (11–13). Noradrenergic degeneration often leads to compensatory changes (12–14) and enhanced responses to norepinephrine (NE) that likely underlie agitation, aggressive behaviors, and sleep disturbance in early AD (14–16). Whereas the noradrenergic system is well recognized as a sensitive target of A β and tau toxicity, our study reveals an unexpected direct etiological role of NE in AD pathogenesis. We report

that A β oligomers at nanomolar concentrations hijack NE-elicited signaling through α_{2A} adrenergic receptor (α_{2A} AR) to activate glycogen synthase kinase 3 β (GSK3 β), resulting in tau hyperphosphorylation. GSK3 β is a prominent tau kinase (17–20) and serves as an integral regulator in the development of AD pathophysiology and cognitive deficits (21–23). Thus, NE/ α_{2A} AR directly mediates A β toxic effects. This NE-dependent mechanism markedly increases the response sensitivity of GSK3 β /tau signaling to A β by nearly two orders of magnitude and provides a possible role for NE in failures of clinical trials targeting A β clearance. Given the enriched expression of α_{2A} AR in noradrenergic neurons, this mechanism may also render this neuronal population selectively vulnerable in AD. Our data obtained from human tissue samples and longitudinal clinical analysis, and two mouse models collectively support hyperactive noradrenergic signaling in AD as a critical element linking A β to the pathogenic GSK3 β /tau cascade that ultimately leads to cognitive impairment.

RESULTS **α_{2A} AR activity is elevated in patients with AD and mouse models**

α_{2A} AR is broadly expressed in both noradrenergic and non-noradrenergic neurons in the brain and controls both NE input and its resulting responses (24, 25). As a member of the G protein-coupled receptor (GPCR) superfamily, α_{2A} AR activates heterotrimeric G proteins to trigger signal transduction. Our pharmacological characterization of α_{2A} AR in postmortem prefrontal cortex (table S1) revealed a significant increase ($P < 0.01$) in α_{2A} AR activity [E_{max}/B_{max} , reflecting maximum G protein activation in response to NE per receptor (26)] in AD cases compared to nondemented, low pathology control subjects (Fig. 1A). Furthermore, our analysis of cases from the National Alzheimer's Coordinating Center (NACC) database (table S2) revealed that usage of clonidine, an α_{2A} AR activator, was associated with worsened cognitive function in patients with cognitive deficits, whereas it had no effect in subjects with normal cognition (Fig. 1B). The adverse effect of clonidine was stronger in patients with more severe dementia

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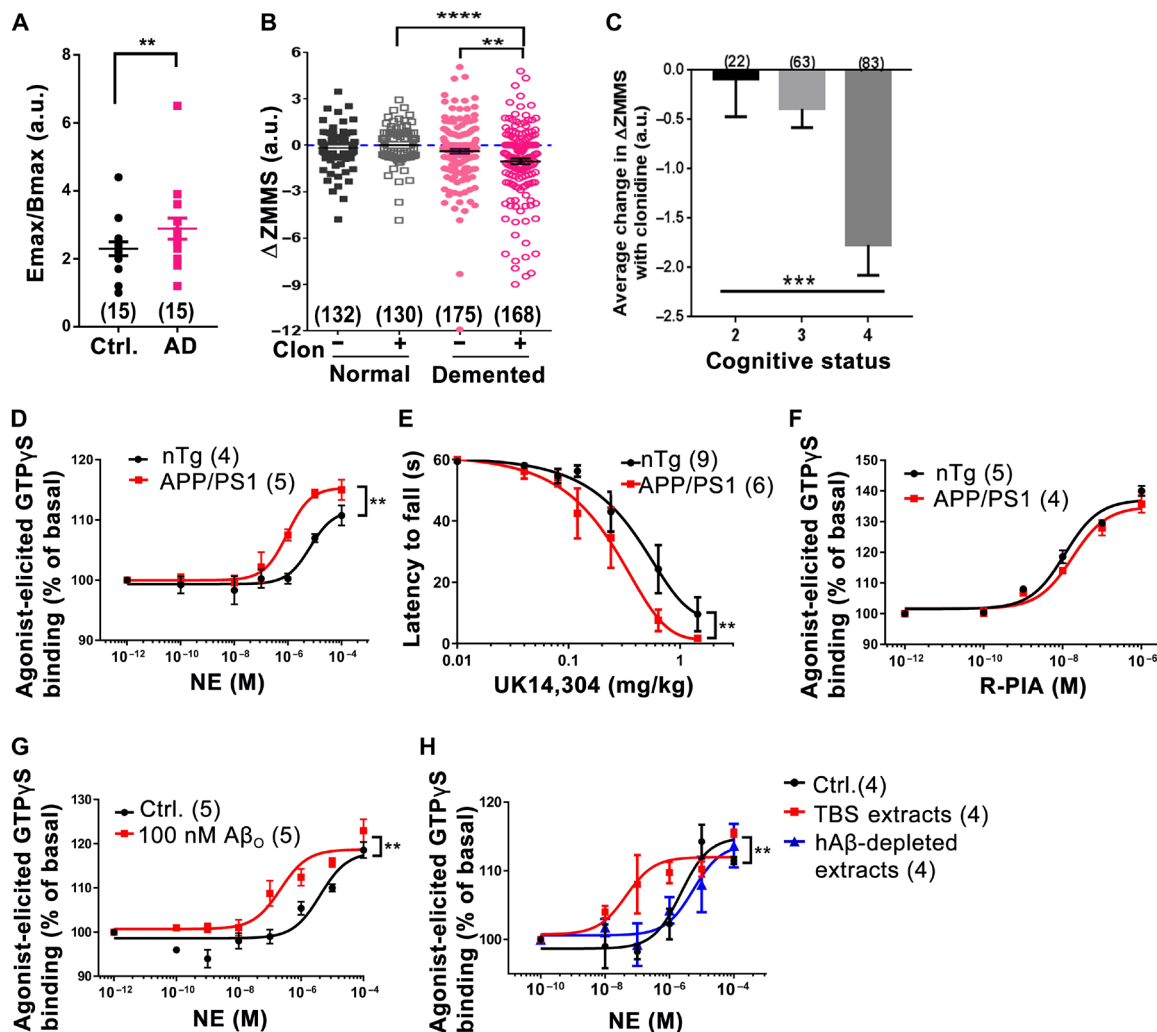


Fig. 1. α_{2A} AR function is enhanced in patients with AD and animal models. (A) Membrane homogenates were prepared from postmortem prefrontal cortical tissues of patients with AD and control subjects. Bmax reflects α_{2A} AR density. Emax reflects maximum α_{2A} AR-mediated G protein activation in response to NE (applied with propranolol and prazosin to selectively activate α_{2A} AR). For each set of experiments, an AD subject and a control subject were analyzed in parallel. $**P < 0.01$ by paired *t* test. (B) Changes in the adjusted z score for the mini-mental state (ZMMS) examination during the time period with or without clonidine usage were analyzed. Δ ZMMS reflects average change in ZMMS in a year. $**P < 0.01$; $****P < 0.0001$, by post hoc Sidak's multiple comparisons test. (C) Average changes in the adjusted ZMMS score in patients with cognitive deficits during the time period with clonidine usage. Cognitive status code: 2, impaired not mild cognitive impairment (MCI); 3, MCI; and 4, dementia. $***P < 0.001$ by one-way ANOVA. (D) Brain homogenates were prepared from nontransgenic (nTg) or APP/PS1 littermates at 7.5 months of age. G protein activation was measured in response to NE (with prazosin and propranolol to selectively activate α_{2A} AR). $**P < 0.01$ by two-way ANOVA. (E) Sedation was measured by rotarod test in response to an α_{2A} AR activator, UK14,304, in nTg, or APP/PS1 littermates at 7.5 to 8 months of age. $**P < 0.01$ by two-way ANOVA. (F) G protein activation in response to an A1R-selective activator, (R)-N6-(1-Methyl-2-phenylethyl)adenosine (R-PIA), in brain homogenates prepared from nTg or APP/PS1 littermates at 7.5 months of age. (G) α_{2A} AR-mediated G protein activation in WT mouse brain homogenates in the presence or absence of $A\beta_o$ (100 nM, monomer equivalent). $**P < 0.01$ by two-way ANOVA. (H) α_{2A} AR-mediated G protein activation in WT mouse brain homogenates in the presence of human TBS extracts with or without $A\beta$ depletion. $**P < 0.01$, TBS extracts versus control by two-way ANOVA. All data are shown as means \pm SEM. a.u., arbitrary units; GTP- γ -S, guanosine 5'-O-(3'-thiotriphosphate).

(Fig. 1C). Combined, these data suggest that α_{2A} AR signaling is hyperactive in patients with AD, and activation of this receptor is detrimental to cognitive function.

We recapitulated the AD-related increase in α_{2A} AR activity in mouse models. We first compared APP(Swe)/PS1 Δ E9 (APP/PS1) transgenic mice (27) and their age-matched nontransgenic littermates at 7.5 months of age, well after initiation of $A\beta$ deposition. There was a leftward shift of the ex vivo NE dose-response curve that indicates enhanced efficiency of α_{2A} AR-mediated G protein activation in the brains of APP/PS1 mice compared to their age-matched non-

transgenic littermates (Fig. 1D). In addition, the α_{2A} AR-elicited sedation response was potentiated in 7.5-month-old APP/PS1 mice (Fig. 1E), suggesting that the increase in G protein activation efficiency by α_{2A} AR indeed results in enhancement of in vivo α_{2A} AR function in experimental AD. G protein activation by another Gi/o-coupled receptor, the adenosine A1 receptor, is not altered in APP/PS1 mice (Fig. 1F), suggesting specific enhancement of α_{2A} AR activity in these mice. Similarly, we observed enhanced efficiency of α_{2A} AR-mediated G protein activation in an independent AD mouse model, *App*^{NL-G-F/NL-G-F} knock-in (APP-KI) (28), at 7.5 months of age

compared to age-matched wild-type (WT) controls (fig. S1). There was no change in α_{2A} AR density in transgenic or KI mice compared to their respective controls (fig. S2).

We found no difference in α_{2A} AR-mediated G protein activation nor α_{2A} AR density between APP/PS1 and nontransgenic mice at 5 weeks of age before the development of A β -related pathology (fig. S3), suggesting that the change in α_{2A} AR response efficiency in 7.5-month-old transgenic mice is likely attributed to A β accumulation in the brain. In support of this notion, addition of A β 42 oligomers (A β _o; 100 nM, monomer equivalent; fig. S4), which are the primary toxic species in AD, sufficiently increased the efficiency of NE to induce G protein activation through α_{2A} AR (Fig. 1G). Furthermore, tris-buffered saline (TBS) extracts from AD prefrontal cortex (AD-TBS extracts), which contain soluble A β oligomers (29),

also increased the efficiency of α_{2A} AR-mediated G protein activation (Fig. 1H and fig. S5).

A β _o acts as an allosteric ligand of α_{2A} AR

The direct effect of A β _o on α_{2A} AR function motivated us to test whether A β _o could bind to α_{2A} AR directly. Flow cytometry assays showed binding of A β _o, but not A β 42 monomers, to cells expressing α_{2A} AR (Fig. 2, A to C). Bound A β _o was colocalized with α_{2A} AR on the cell surface (Fig. 2D). Binding of A β _o to immunopurified α_{2A} AR showed a saturable curve with a dissociation constant (K_d) less than 30 nM (monomer equivalent concentration of total A β 42 peptide, Fig. 2E). We could not detect substantial binding of A β _o to two other GPCRs, α_{2C} AR (another α_2 AR subtype), and adenosine A1 receptor (Fig. 2F and fig. S6), demonstrating the specificity of the A β _o- α_{2A} AR interaction.

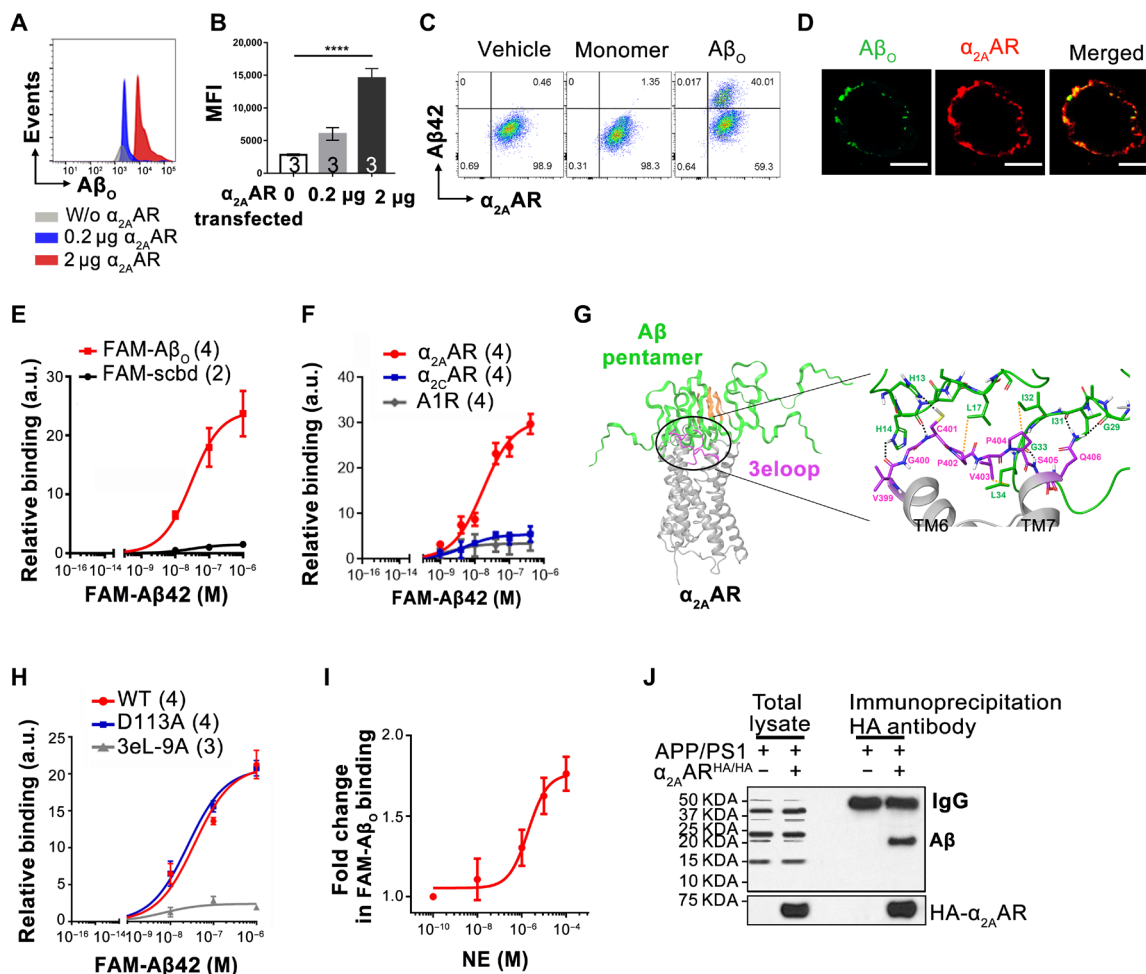


Fig. 2. A β _o binds to an allosteric site of α_{2A} AR with nanomolar affinity. (A and B) Human embryonic kidney (HEK) cells transfected with the empty vector or hemagglutinin (HA)-tagged α_{2A} AR were incubated with A β _o for 30 min. A β _o bound to the surface of cells was detected by flow cytometry assays. **** P < 0.0001 by one-way ANOVA in (B). (C) Flow cytometry assays were performed with cells expressing HA- α_{2A} AR after incubation with vehicle, monomer, or oligomer A β . (D) A β _o and HA- α_{2A} AR were detected by immunocytochemistry. Scale bars, 5 μ m. (E) HA- α_{2A} AR was immunopurified from HEK cells and incubated with increasing amounts of 5-Carboxyfluorescein (FAM)-labeled A β _o or scrambled (scdbd) A β 42 peptide. (F) Saturation binding curves of FAM-A β _o to different receptors expressed on the surface of intact HEK cells. (G) The docked A β _o- α_{2A} AR complex model. Green, A β pentamer with hydrophobic C termini of monomers indicated in orange. Purple, the 3eL of α_{2A} AR. Dashed black lines and orange lines indicate hydrogen bonds and hydrophobic contacts, respectively. (H) Binding of FAM-A β _o to WT or α_{2A} AR mutants, as indicated, expressed on the cell surface. (I) Binding of FAM-A β _o (20 nM, monomer equivalent) to immunopurified α_{2A} AR in the presence of increasing concentrations of NE. All data are shown as means \pm SEM. (J) Total brain lysates prepared from APP/PS1 or APP/PS1, α_{2A} AR^{HA/HA} mice were subjected to coimmunoprecipitation assays using an HA antibody. The α_{2A} AR^{HA/HA} allele harbors an HA tag at the N terminus of the endogenous α_{2A} AR locus. APP/PS1 mouse brains were used as a negative control. Representative blots from multiple experiments are shown. IgG, immunoglobulin G; IP, immunoprecipitation.

We next determined the nature of A β _O binding to α _{2A}AR. Our *in silico* docking result indicated that A β _O likely binds to the third extracellular loop (3eL) of α _{2A}AR (Fig. 2G). Alanine mutations of nine amino acids (amino acids 399 to 406) in the 3eL (3eL-9A) abolished A β _O binding to the receptor (Fig. 2H and fig. S7) without affecting binding of an orthosteric ligand, RX821002 (fig. S8). Conversely, a mutation at D113 of α _{2A}AR (D113A) that eliminates binding of orthosteric ligands (fig. S8) (30) did not alter binding of A β _O to α _{2A}AR (Fig. 2H). These data demonstrate that A β _O binds to an allosteric site of α _{2A}AR involving the 3eL. Furthermore, NE enhanced the binding affinity of A β _O to α _{2A}AR in a dose-dependent and saturable manner (Fig. 2I). These results, along with the fact shown above that A β _O enhanced the potency of NE (reflecting its binding affinity to the receptor) to elicit α _{2A}AR-mediated G protein activation, clearly demonstrate the reciprocal nature of the regulation between a GPCR orthosteric ligand (NE) and an allosteric ligand (A β _O). Moreover, our data suggest that A β _O actions can be sensitized by an endogenous neurotransmitter (NE). To examine the endogenous interaction between A β _O and α _{2A}AR, we crossbred the APP/PS1 line with the HA-tagged α _{2A}AR KI line (α _{2A}AR^{HA/HA}) that we generated previously (31) to acquire APP/PS1, α _{2A}AR^{HA/HA} mice. Using these mice, we detected stable complex formation between the endogenous α _{2A}AR and A β _O in the brain (Fig. 2J).

A β _O binding to α _{2A}AR redirects receptor signaling to activate the GSK3 β /tau cascade

Binding of an allosteric ligand to a GPCR often alters signaling through the same receptor (32, 33). We therefore searched for A β _O-induced changes in α _{2A}AR signaling using protein kinase arrays. Among the kinases tested, we found a change in GSK3 β phosphorylation at Ser⁹ (indicating an increase in activity) ($P < 0.05$; Fig. 3A and fig. S9). In cultured primary neurons, we observed a significant reduction ($P < 0.01$) in GSK3 β phosphorylation at Ser⁹ only in cells cotreated with A β _O and NE but not in cells treated with either agent alone (Fig. 3, B and C). When coapplied with clonidine, A β _O sufficiently induced GSK3 β dephosphorylation/activation at a concentration of 20 nM (monomer equivalent; Fig. 3, B and D). This amount is less than 1% of the concentration required for GSK3 β activation in neurons by A β _O alone (34, 35). Furthermore, naturally secreted oligomeric A β at nanomolar concentrations (36) also induced GSK3 β dephosphorylation/activation in neurons in the presence of clonidine (fig. S10). Concurrent with GSK3 β activation in cells cotreated with NE and A β _O, we observed a significant increase ($P < 0.01$) in tau hyperphosphorylation at AD-relevant sites, Ser²⁰² and Thr²⁰⁵, detected by AT8 antibody (Figs. 3E and 4, A and C, and fig. S11). This change in tau could not be detected in cells where GSK3 β expression was suppressed by small interfering RNAs (siRNAs) (Fig. 3E and fig. S11), suggesting that GSK3 β activation is required for tau hyperphosphorylation in response to NE and A β _O cotreatment. Together, these data demonstrate that A β aberrantly redirects NE-induced α _{2A}AR signaling to GSK3 β activation and subsequent tau hyperphosphorylation. This NE/ α _{2A}AR-dependent pathway can increase the response sensitivity of GSK3 β /tau signaling to A β by two orders of magnitude.

We further examined the role of endogenous α _{2A}AR activation by NE in A β -induced GSK3 β /tau signaling *in vivo*. A β _O (100 pmol, monomer equivalent) or vehicle was microinjected bilaterally into the dorsal hippocampus of WT C57BL/6 mice, which then received treatment with either saline, idazoxan (an α _{2A}AR blocker), or lithium (a GSK3 β blocker). A β _O injection induced a decrease in GSK3 β

phosphorylation and a concurrent increase in tau hyperphosphorylation (detected by AT8 antibody) in dorsal hippocampi when compared to vehicle sham controls (Fig. 3, F to H). A β _O-induced tau hyperphosphorylation was diminished by lithium treatment (Fig. 3, F and H), indicating an essential role of GSK3 β in this process. When α _{2A}AR was blocked by idazoxan, A β _O-induced changes in GSK3 β and tau were abolished (Fig. 3, F to H). These data suggest that endogenous α _{2A}AR activation is required for A β -induced GSK3 β /tau signaling *in vivo*, providing strong evidence for an essential role of noradrenergic signaling in A β -induced tau hyperphosphorylation.

To validate that A β _O acts through allosteric binding to α _{2A}AR to activate the GSK3 β /tau signaling, we performed experiments with cells expressing the 3eL-9A mutant α _{2A}AR, which cannot interact with A β _O (Fig. 4A). Mutations in the 3eL did not affect binding of orthosteric ligands to α _{2A}AR (fig. S8), and this mutant receptor was still able to mediate G protein signaling in response to NE (Fig. 4D). However, in cells expressing the 3eL-9A mutant receptor, cotreatment with A β _O and NE failed to alter either GSK3 β or tau phosphorylation (Fig. 4, A to C). These data suggest that the allosteric binding of A β _O to the 3eL of α _{2A}AR is required for activation of the pathogenic GSK3 β /tau cascade. Collectively, our data reveal a previously unappreciated molecular mechanism, namely, hijacking NE/ α _{2A}AR signaling, that enables nanomolar concentrations of extracellular A β to activate the pathogenic GSK3 β /tau cascade and that this pathway can be effectively blocked by α _{2A}AR inhibitors (fig. S12).

Blockade of α _{2A}AR reduces GSK3 β activation and tau hyperphosphorylation and ameliorates AD-related pathological and cognitive deficits

Our results reveal an NE/ α _{2A}AR-dependent mechanism connecting A β to the pathogenic GSK3 β /tau cascade. We therefore hypothesized that blocking α _{2A}AR in the presence of A β pathology would have therapeutic potential. To test this, we treated APP/PS1 mice with idazoxan for 8 weeks starting at 7.5 to 8 months of age when A β plaques were present, and α _{2A}AR activity was enhanced. Compared to saline treatment, idazoxan reversed hyperactivation of GSK3 β in APP/PS1 mouse brains (Fig. 5, A and B), providing additional support for the critical role of α _{2A}AR in mediating A β -induced GSK3 β activation *in vivo*. In the cerebral cortex of idazoxan-treated APP/PS1 mice, the extent of A β load was lower than that in saline-treated mice (Fig. 5, C and D), indicating that blocking α _{2A}AR slows the progression of A β pathology. This effect likely results both from the reduction in GSK3 β activity, given that GSK3 β can promote A β generation (37), and from regulation of sorting-related receptor with A repeats (SorLA)-mediated APP trafficking, as we previously reported (38). Similarly, idazoxan treatment of APP-KI mice also reduced A β plaque load (fig. S13) and GSK3 β activation (fig. S14) in the brain. We further examined another important feature of AD pathology, microglial activation (39). Idazoxan treatment decreased the density of Iba-1-positive microglial cells (Fig. 5, E and F), suggesting reduction of neuroinflammation.

In the cerebral cortex of APP/PS1 mice, we detected accumulations of pretangle phospho-tau clusters (detected by AT8 antibody) in areas with A β accumulation (detected by an A β antibody) (Fig. 5G), and the intensity of AT8 staining positively correlated with the size of A β depositions (Fig. 5H), consistent with the idea that the A β plaque environment promotes pathological changes in tau (40). In idazoxan-treated APP/PS1 mice, the appearance and intensity of AT8-positive signals were markedly reduced compared to those in

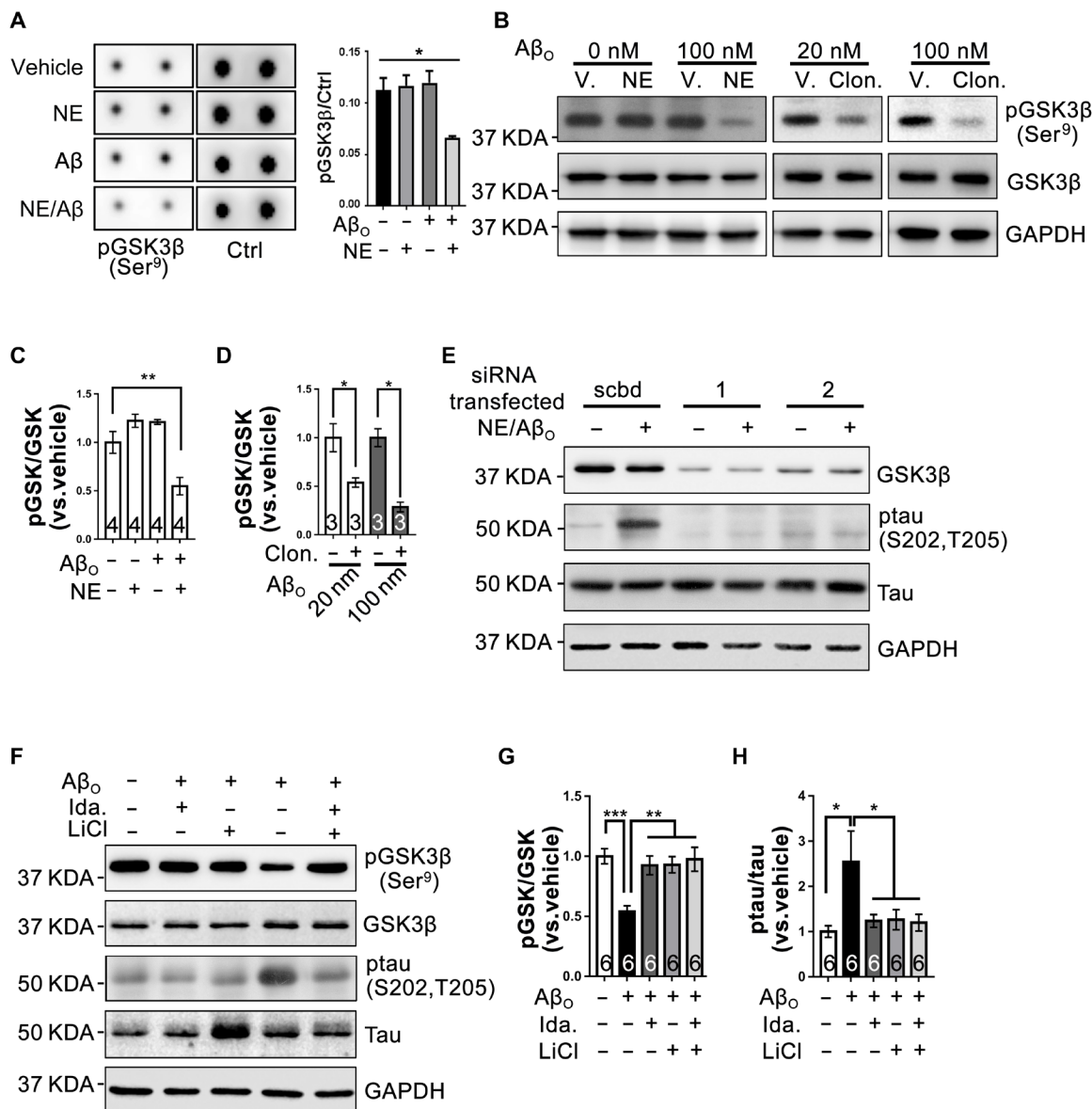


Fig. 3. Aβ₀ redirects α_{2A}AR signaling to activation of the GSK3β/tau cascade. (A) Representative blots and quantitation of protein kinase arrays. Array blots were incubated with lysates from Neuro2A cells expressing WT α_{2A}AR with treatment as indicated. NE (400 nM) was applied with prazosin and propranolol to selectively activate α_{2A}AR. Ctrl, positive controls for array blotting. **P* < 0.05 by one-way ANOVA. (B) Primary cortical neurons (14 days in vitro) were stimulated as indicated for 30 min. V, vehicle; Clon, clonidine (1 μM). Representative Western blots of phospho-GSK3β (pGSK3β), total GSK3β, and glyceraldehyde-3-phosphate dehydrogenase (GAPDH). (C and D) Quantitation of changes in the ratio of pGSK3β to GSK3β. ***P* < 0.01 by one-way ANOVA Tukey's multiple comparisons. **P* < 0.05 by unpaired *t* test. (E) Neuro2A cells were cotransfected with WT α_{2A}AR and a siRNA against GSK3β or scrambled (scbd) siRNA. Representative blots of tau phosphorylation are shown. (F to H) Mice that received bilateral intrahippocampal injection of Aβ₀ (100 pmol, monomer equivalent, each side) or vehicle were treated intraperitoneally with saline, idazoxan (3 mg/kg), lithium (300 mg/kg), or idazoxan and lithium. Twenty-four hours later, hippocampal lysates were analyzed by Western blot. Representative blots (F) and quantitation (G and H) of GSK3β and tau phosphorylation are shown. **P* < 0.05, ***P* < 0.01, ****P* < 0.001 by one-way ANOVA Tukey's multiple comparisons. All data are shown as means ± SEM.

saline-treated littermates (Fig. 5, G to I). Furthermore, idazoxan treatment reduced tau hyperphosphorylation induced by the same amount of Aβ deposition (Fig. 5, H and I), suggesting that blockade of α_{2A}AR effectively alleviates Aβ-induced tau pathology. In APP-KI mice, idazoxan treatment also reduced tau hyperphosphorylation in the brain (fig. S14).

Reduced Aβ pathology and tau hyperphosphorylation would result in enhanced preservation of cognitive function. Saline-treated APP/PS1 mice showed clear deficits in the Morris water maze task

when compared to their age-matched nontransgenic littermate controls, whereas idazoxan-treated APP/PS1 mice performed significantly better (*P* < 0.01; Fig. 6, A and B). Despite the presence of a substantial Aβ burden (Fig. 5C), idazoxan-treated APP/PS1 mice behaved similarly to their nontransgenic littermates on the last day of training (Fig. 6, A and B). Idazoxan treatment did not alter the baseline activity or anxiety (figs. S15 and S16). Cognitive changes in APP-KI mice were also mitigated by idazoxan treatment. Compared to age-matched WT controls, APP-KI mice showed reduced latency to the

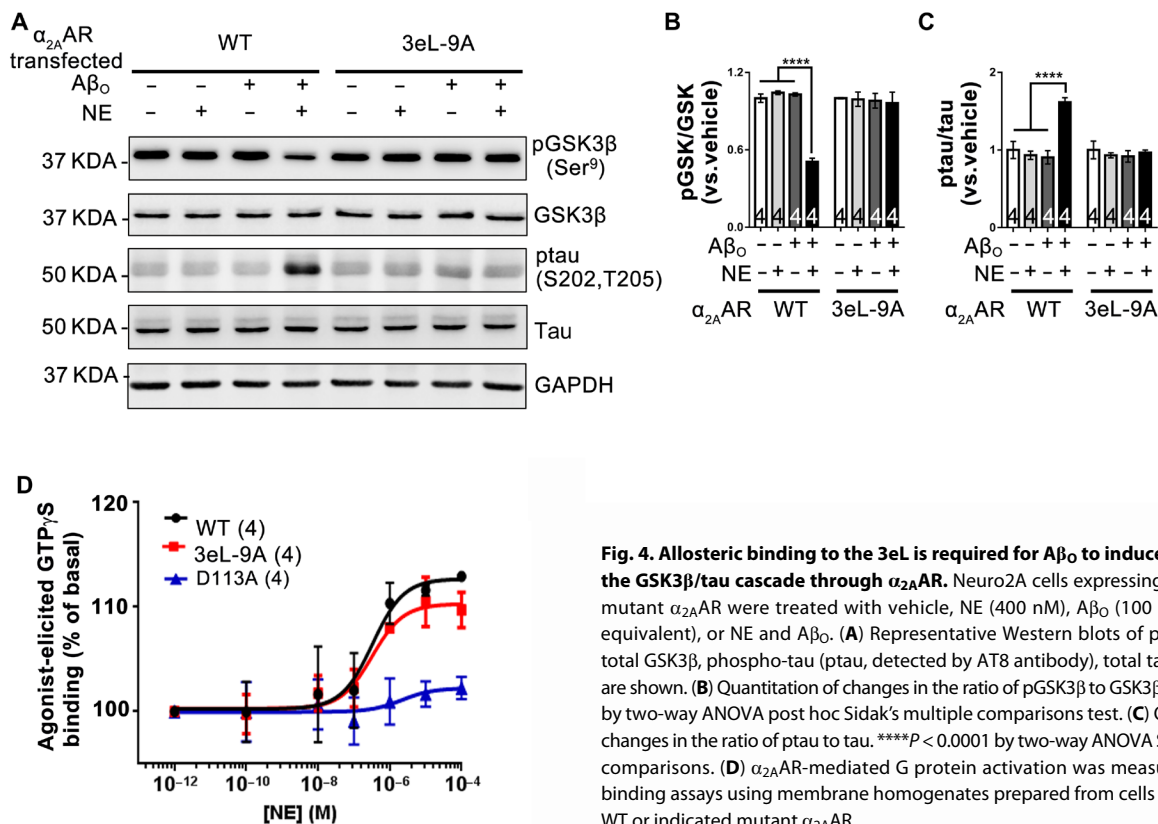


Fig. 4. Allosteric binding to the 3eL is required for $A\beta_O$ to induce activation of the GSK3 β /tau cascade through $\alpha_{2A}AR$. Neuro2A cells expressing WT or 3eL-9A mutant $\alpha_{2A}AR$ were treated with vehicle, NE (400 nM), $A\beta_O$ (100 nM, monomer equivalent), or NE and $A\beta_O$. **(A)** Representative Western blots of pGSK3 β at Ser⁹, total GSK3 β , phospho-tau (ptau, detected by AT8 antibody), total tau, and GAPDH are shown. **(B)** Quantitation of changes in the ratio of pGSK3 β to GSK3 β . **** P < 0.0001 by two-way ANOVA post hoc Sidak's multiple comparisons test. **(C)** Quantitation of changes in the ratio of ptau to tau. **** P < 0.0001 by two-way ANOVA Sidak's multiple comparisons. **(D)** $\alpha_{2A}AR$ -mediated G protein activation was measured by GTP γ S binding assays using membrane homogenates prepared from cells expressing the WT or indicated mutant $\alpha_{2A}AR$.

dark on day 2 in passive avoidance task (Fig. 6C), which was normalized by idazoxan treatment (Fig. 6D). These behavioral changes were not due to acute drug effects, but are interpreted as a result of the reduction in GSK3 β /tau signaling by idazoxan treatment because a 1-week drug washout period was incorporated before testing the mice. These data collectively demonstrate that blocking NE signaling through $\alpha_{2A}AR$ is an effective strategy to ameliorate pathological and cognitive deficits associated with $A\beta$.

DISCUSSION

Our current study provides strong evidence for an essential role of noradrenergic signaling in $A\beta$ proteotoxicity. We show that $A\beta_O$ can hijack NE-elicited signaling through $\alpha_{2A}AR$ to induce activation of the pathogenic GSK3 β /tau cascade (fig. S12), resulting in tau hyperphosphorylation and accelerated cognitive decline. This NE/ $\alpha_{2A}AR$ -dependent mechanism allows $A\beta_O$ to induce GSK3 β activation at a concentration as low as 1% of that required for GSK3 β activation by $A\beta_O$ alone and thus could be engaged in early stages of AD when $A\beta$ concentrations are low. Normal physiological concentrations of $A\beta$ are in picomolar ranges (41, 42), and oligomers start to form when $A\beta$ concentrations reach a critical aggregation concentration of 90 nM (43). At this concentration, $A\beta_O$ can sufficiently induce the pathogenic GSK3 β /tau cascade through the mechanism identified here, providing a route for $A\beta$ to initiate the disease process. We thus speculate that the noradrenergic system, $\alpha_{2A}AR$ in particular, could play a critical role in establishing the $A\beta$ -dependent tipping point at which hyperphosphorylation of tau accelerates. Interventions targeting this NE/ $\alpha_{2A}AR$ -dependent mechanism of $A\beta$ action

would be helpful in slowing or even halting the transition from normal physiology to the earliest stages of disease. Our study may also inform interpretation of failed clinical trials targeting $A\beta$. Considering that $A\beta$ in human AD brains can reach micromolar concentrations (44), it would be difficult to reduce $A\beta$ to below nanomolar concentrations to prevent activation of the $\alpha_{2A}AR$ /GSK3 β /tau cascade.

We show that $\alpha_{2A}AR$ activity is enhanced in AD, which would further sensitize neurons to $A\beta$ -induced tau pathology and cognitive dysfunction. This notion aligns with results reported here from unbiased epidemiological analysis of the NACC database and preclinical studies using two mouse models of experimental AD. Given that $\alpha_{2A}AR$ is highly expressed in LC noradrenergic neurons, the $A\beta$ / $\alpha_{2A}AR$ /GSK3 β /tau cascade also provides a possible mechanism for these neurons to be exquisitely vulnerable in AD.

Our preclinical studies demonstrate that pharmacologically blocking endogenous NE/ $\alpha_{2A}AR$ signaling can effectively reduce activation of the GSK3 β /tau cascade, resulting in mitigation of behavioral deficits. $\alpha_{2A}AR$ blockers such as idazoxan have been developed for use in other disorders, and repurposing these drugs could be a potentially effective, readily available strategy for AD treatment. In addition, our data suggest that the $A\beta$ - $\alpha_{2A}AR$ interaction represents an attractive disease-specific therapeutic target for AD because the $\alpha_{2A}AR$ /GSK3 β /tau cascade can only be activated in the presence of $A\beta$ oligomers. Directly targeting the $A\beta$ - $\alpha_{2A}AR$ interface would not interfere with normal $\alpha_{2A}AR$ functions and therefore be less likely to result in complications associated with an extended dosing period necessary for AD treatment. Furthermore, from a pharmacological perspective, simultaneously targeting both the orthosteric and allosteric binding sites would create a synergistic effect on receptor-mediated

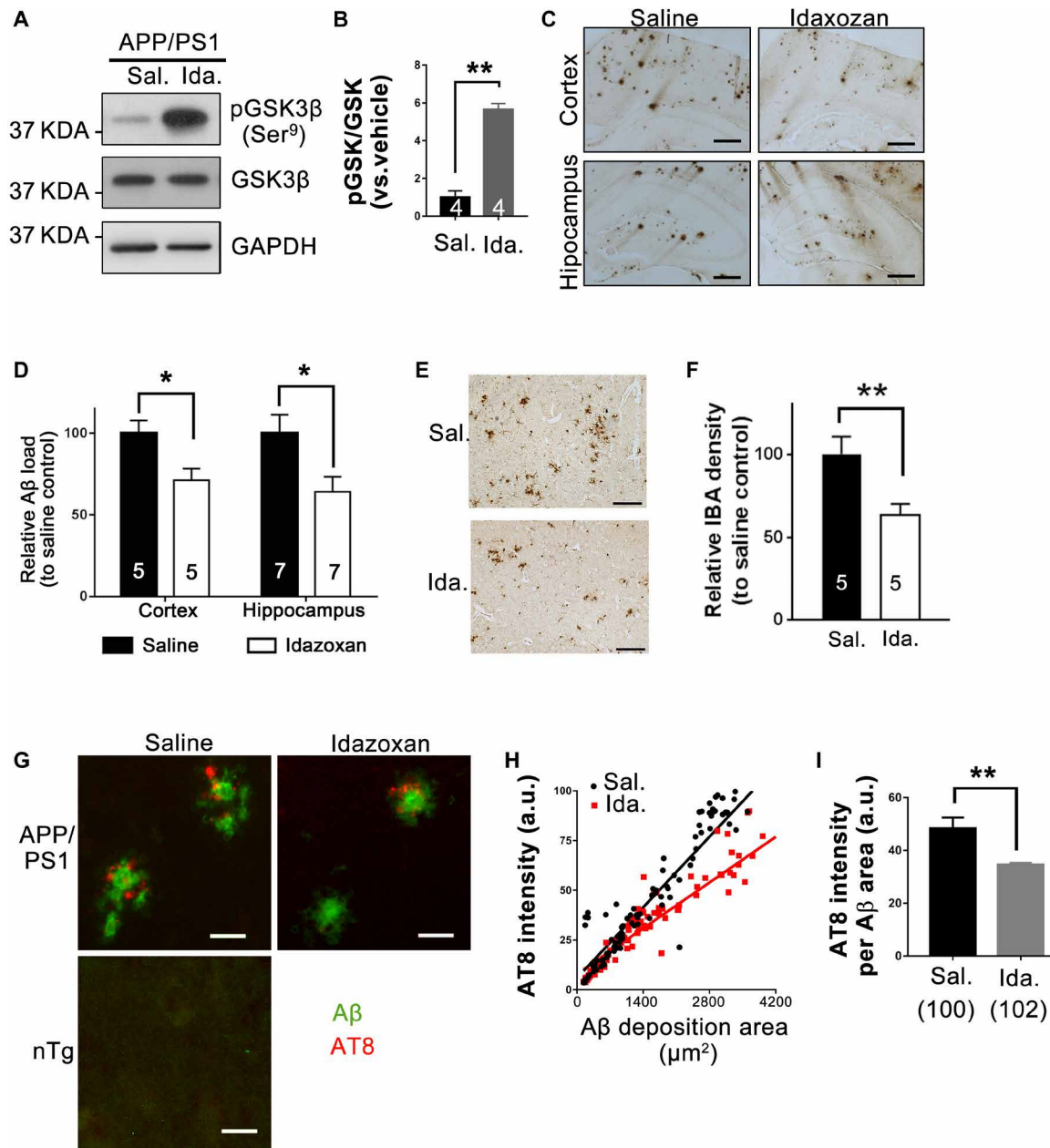


Fig. 5. Blocking α_{2A} AR in AD model mice with profound A β pathology reduces GSK3 β activity, amyloid pathology, and tau hyperphosphorylation. Eight-month-old APP/PS1 and nontransgenic (nTg) littermate mice were treated with saline or idazoxan for 8 weeks, followed by a 1-week washout period. **(A)** Representative Western blots and **(B)** quantitation of phospho-GSK3 β (pGSK3 β) at Ser⁹ and total GSK3 β in total cortical lysates. ****** P < 0.01 by unpaired Student's t test. **(C)** Representative images and **(D)** quantitation of A β plaques (detected by 6E10 antibody) in the cerebral cortex and hippocampus of APP/PS1 mice subjected to treatments indicated. Scale bars, 500 μ m. ***** P < 0.05 by unpaired t test. **(E)** Representative images and **(F)** quantitation of microglial cells (detected by Iba-1 antibody) in the cerebral cortex of APP/PS1 mice subjected to treatments were indicated. Scale bars, 100 μ m. ****** P < 0.01 by unpaired t test. **(G)** Representative images of AT8 (for hyperphosphorylated tau) and an A β antibody staining in the cortex of nTg and APP/PS1 mice after the indicated treatment. Scale bars, 20 μ m. **(H)** Quantitation of the intensity of AT8 signals plotted against the area of A β accumulations in the cortex. $r^2 = 0.84$. Slope values for saline and idazoxan groups are 34.29 (± 1.519) and 22.98 (± 1.017), respectively. **(I)** Relative AT8 intensity normalized against the corresponding area of A β depositions. ****** P < 0.01 by unpaired t test.

responses (45). Thus, the combined use of α_{2A} AR blockers (targeting the orthosteric site) and drugs that reduce A β load (decreasing the allosteric binding of A β to α_{2A} AR) may lead to an enhanced therapeutic effect.

We are aware of limitations of our study. Our analysis of the clonidine effect on cognition in human patients is not a controlled

study, and the sample size is relatively small. Nonetheless, our unbiased epidemiological analysis of the longitudinal clinical data supports the notion that chronic α_{2A} AR activation exacerbates AD disease progression in human patients. It would also be useful to analyze the effect of α_{2A} AR blockers in patients. Unfortunately, the sample size of subjects using other α_{2A} AR agonists or antagonists in the

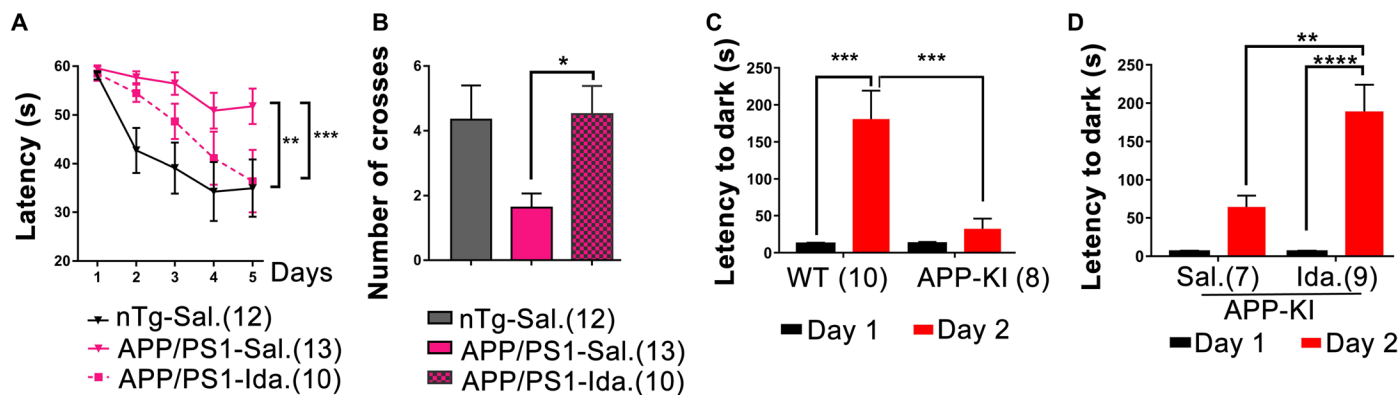


Fig. 6. Blocking $\alpha_{2A}AR$ in AD model mice with profound $A\beta$ pathology ameliorates cognitive deficits. (A) Measurement of escape latency on each day in Morris water maze tests in APP/PS1 and nTg mice. *** $P < 0.001$, saline-treated APP/PS1 versus nTg mice; ** $P < 0.01$, saline-treated versus idazoxan-treated APP/PS1 mice by two-way ANOVA. (B) Quantitation of the number of crosses of the target quadrant in probe trial. * $P < 0.05$ by one-way ANOVA post hoc Tukey's multiple comparisons test. (C) Measurement of escape latency to the dark side in passive avoidance tests in 8-month-old WT and APP-KI mice. *** $P < 0.001$ by two-way ANOVA Tukey's multiple comparisons test. (D) Measurement of escape latency to the dark side in passive avoidance tests in 8-month-old APP-KI mice treated with saline or idazoxan. ** $P < 0.01$ and **** $P < 0.0001$ by two-way ANOVA Tukey's multiple comparisons test. All data are shown as means \pm SEM.

NACC database is too small for statistical tests. Another limitation of the study is that a homology model of $\alpha_{2A}AR$ was used in the in silico docking study, as the $\alpha_{2A}AR$ crystal structure is not available. A precise view of the structural base of the $A\beta$ - $\alpha_{2A}AR$ interaction would be necessary for the design of compounds to disrupt the interaction interface.

In summary, our current study demonstrates that $A\beta$ rewires NE signaling to induce activation of the pathogenic GSK3 β /tau pathway, providing new insights into mechanisms underlying $A\beta$ proteotoxicity, which have strong implications for the interpretation of $A\beta$ clearance trial results and future drug design.

MATERIALS AND METHODS

Study design

The overall goal of our study is to address the potential role of the brain noradrenergic system in AD pathogenesis. We focused on $\alpha_{2A}AR$, a key component of the noradrenergic system. We first examined the potential disease relevance of this receptor to AD using human tissue samples and longitudinal clinical data and then recapitulated the AD-related increase in $\alpha_{2A}AR$ activity in two independent AD mouse models. Next, we determined properties of $A\beta$ oligomer ($A\beta_0$) binding to $\alpha_{2A}AR$ using combined biochemical, cell biological, pharmacological, and computational methods. We then investigated the biological consequence of the $A\beta_0$ - $\alpha_{2A}AR$ interaction on intracellular signaling in neuronal cells and in the brain. Last, we performed preclinical studies to explore the therapeutic potential of blocking $\alpha_{2A}AR$ in ameliorating AD-related pathological and cognitive deficits. Throughout the study, we exploited an interdisciplinary approach, used multiple technical controls, and included both technical replicates and biological repeats in our assays. Littermate mice were randomly assigned to different treatment groups. Experimenters were blinded with phenotypes or treatments in animal studies. Sample sizes were determined on the basis of previous experience with similar studies. The number of samples indicated in the figure legends reflects independent biological repeats. Conclusions were drawn on the basis of careful statistical analyses.

Statistical analysis

All statistical analyses were performed using GraphPad Prism 7.0 software. In general, Student's *t* test was performed to determine differences between two groups, and one-way or two-way analysis of variance (ANOVA) was performed to determine variations in multiple groups with one or two variances. For Fig. 1A, age- and gender-matched controls and AD samples were paired and analyzed in parallel, and paired *t* test was performed to determine the difference between the two groups. For Fig. 1C, one-way ANOVA was performed, and for the rest of the panels in Fig. 1, two-way ANOVA was used. For Fig. 3 (C, G, and H), one-way ANOVA was performed, and post hoc Tukey's multiple comparisons were used to determine the difference between two groups. For Fig. 4 (B and C), two-way ANOVA and post hoc Sidak's multiple comparisons were performed. For Fig. 6 (A, C, and D), two-way ANOVA and post hoc Tukey's multiple comparisons were performed. For all statistical tests, $P < 0.05$ was considered statistically significant. Data are presented as means \pm SEM. Non-linear regression curve fit for saturation binding and dose-response curves was also performed using GraphPad Prism.

SUPPLEMENTARY MATERIALS

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Materials and Methods

Fig. S1. $\alpha_{2A}AR$ -mediated G protein activation in brain homogenates prepared from WT or APP-KI mice at 7.5 months of age.

Fig. S2. $\alpha_{2A}AR$ density measured by radioligand-binding assays in AD models.

Fig. S3. $\alpha_{2A}AR$ -mediated G protein activation and receptor density tested in brain homogenates prepared from nTg or APP/PS1 mice at 5 weeks of age.

Fig. S4. Profiling of $A\beta_{42}$ peptide oligomerization by fluorescent size-exclusion chromatography.

Fig. S5. $\alpha_{2A}AR$ -mediated G protein activation in WT mouse brain homogenates in the presence of human TBS extracts with or without $A\beta$ depletion.

Fig. S6. Cell-surface expression of HA-tagged receptors tested by fluorescence-activated cell sorting.

Fig. S7. $A\beta_0$ was detected on the surface of cells expressing WT but not 3E-L-9A mutant $\alpha_{2A}AR$.

Fig. S8. Binding of an orthosteric ligand to WT or mutant $\alpha_{2A}AR$.

Fig. S9. Full blots of the AKT pathway phosphorylation arrays.

Fig. S10. Naturally secreted oligomeric $A\beta$ induced GSK3 β dephosphorylation/activation in neurons in the presence of clonidine.

Fig. S11. Quantitation of tau phosphorylation and GSK3 β expression.

Fig. S12. Proposed model of $A\beta$ hijacking NE signaling through $\alpha_{2A}AR$ to induce activation of GSK3 β /tau cascade.

Fig. S13. Idazoxan treatment reduces A β pathology in APP-KI mouse brains.

Fig. S14. Idazoxan treatment reduces GSK3 β activity and tau hyperphosphorylation in APP-KI mouse brains.

Fig. S15. Open-field and elevated zero maze tests in nTg and APP/PS1 mice.

Fig. S16. Open-field and elevated zero maze tests in APP-KI mice.

Table S1. Information of human samples used in Fig. 1A.

Table S2. Extracted data used in Fig. 1 (B and C).

Table S3. Information of antibodies used in this study.

Data file S1. Raw data.

References (27–29, 31, 38, 46–65)

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β -amyloid redirects norepinephrine signaling to activate the pathogenic GSK3 β /tau cascade

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The noradrenergic link

Norepinephrine (NE) and the noradrenergic system play a main role in cognition and noradrenergic changes have been documented in patients with Alzheimer's disease (AD). However, the role of NE in AD has not been completely elucidated. Here, Zhang *et al.* show that β -amyloid oligomers are allosteric ligands of the α 2A adrenergic receptor (α 2AAR) and modulate NE signaling, redirecting the pathway toward GSK3 β activation and subsequent tau hyperphosphorylation. Blocking α 2AAR reduced tau phosphorylation and ameliorated pathological and cognitive abnormalities in AD mouse models.

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