# $\alpha_{2A}$ adrenergic receptor promotes amyloidogenesis through disrupting APP-SorLA interaction

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**Accumulation of amyloid β (Aβ) peptides in the brain is the key pathogenic factor driving Alzheimer's disease (AD). Endocytic sorting of amyloid precursor protein (APP) mediated by the vacuolar protein sorting (Vps10) family of receptors plays a decisive role in controlling the outcome of APP proteolytic processing and Aβ generation. Here we report for the first time to our knowledge that this process is regulated by a G protein-coupled receptor, the $\alpha_{2A}$ adrenergic receptor ($\alpha_{2A}$AR). Genetic deficiency of the $\alpha_{2A}$AR significantly reduces, whereas stimulation of this receptor enhances, Aβ generation and AD-related pathology. Activation of $\alpha_{2A}$AR signaling disrupts APP interaction with a Vps10 family receptor, sorting-related receptor with A repeat (SorLA), in cells and in the mouse brain. As a consequence, activation of $\alpha_{2A}$AR reduces Golgi localization of APP and concurrently promotes APP distribution in endosomes and cleavage by β secretase. The $\alpha_{2A}$AR is a key component of the brain noradrenergic system. Profound noradrenergic dysfunction occurs consistently in patients at the early stages of AD. $\alpha_{2A}$AR-promoted Aβ generation provides a novel mechanism underlying the connection between noradrenergic dysfunction and AD. Our study also suggests $\alpha_{2A}$AR as a previously unappreciated therapeutic target for AD. Significantly, pharmacological blockade of the $\alpha_{2A}$AR by a clinically used antagonist reduces AD-related pathology and ameliorates cognitive deficits in an AD transgenic model, suggesting that repurposing clinical $\alpha_2$AR antagonists would be an effective therapeutic strategy for AD.**

adrenergic receptor | amyloid | processing | SorLA | sorting

Excess amyloid β (Aβ) peptides in the brain are a neuropathological hallmark of Alzheimer's disease (AD) and are generally accepted as the key pathogenic factor of the disease (1). Aβ is generated by two sequential cleavages of amyloid precursor protein (APP) by β and γ secretase, whereas cleavage by α secretase within the Aβ domain precludes Aβ generation (2, 3). APP and the secretases undergo endocytic sorting into various organelles, such as the trans-Golgi network, the plasma membrane, and endosomes (2–6). The initial step of APP processing by α versus β secretase preferentially occurs in distinct compartments of the cell. Although α secretase-mediated cleavage of APP occurs on the plasma membrane, β secretase primarily interacts with and cleaves APP in endosomes (2–6). Therefore, endocytic sorting of APP into different membranous compartments, causing it to coreside or avoid a particular secretase, plays a decisive role in APP proteolytic processing. Consistent with this notion, abnormalities of the endocytic pathway have been found to precede Aβ deposition in late-onset AD (7).

Retrograde sorting of APP from endosomes to trans-Golgi network mediated by the vacuolar protein sorting-10 (Vps10) family proteins and the retromer complex represents a critical mechanism to prevent amyloidogenic processing of APP (8–10) and has recently emerged as a potential target for therapeutic intervention (11). In particular, the sorting-related receptor with A repeat (SorLA) directs retrograde transport of APP to trans-Golgi network by binding to both APP and the retromer complex (12, 13) and retains APP in the Golgi (14), thus preventing its proteolytic processing. A connection between SorLA and AD was first revealed in patients with late-onset AD, in whom the levels of SorLA at the steady state are markedly reduced (15). Further human genetic studies identified variations of *SORL1* (the gene encoding SorLA) resulting in a lower level of expression that are associated with late-onset sporadic AD (12, 16, 17). Moreover, nonsense and missense mutations of *SORL1* cause autosomal dominant early-onset AD (18), supporting an etiological role of SorLA in AD. The function of SorLA in inhibiting Aβ production is confirmed by mouse genetic studies showing that loss of SorLA significantly increases Aβ levels in the brain (14) and enhances AD-related early pathology (19). Despite the importance of SorLA-dependent APP sorting in controlling Aβ metabolism and AD pathogenesis, how this process may be targeted by extracellular stimuli, such as neurotransmitters and hormones, to modulate amyloidogenesis remains largely unstudied.

The $\alpha_{2A}$ adrenergic receptor (AR) belongs to the G protein-coupled receptor (GPCR) superfamily and is a crucial component of the brain noradrenergic (NA) system, controlling both NA input to the cerebral cortex and the resulting response in this brain region (20). Profound dysfunction of the NA system consistently occurs at the early stage of AD (21), raising the possibility of involvement of the $\alpha_{2A}$AR in AD pathogenesis. Here we report for the first time to our knowledge that $\alpha_{2A}$AR signaling regulates SorLA-dependent APP sorting and promotes amyloidogenic processing of APP by beta-site amyloid precursor

## Significance

**Endocytic sorting of amyloid precursor protein (APP) governed by the vacuolar protein sorting (Vps10) family of receptors plays a decisive role in controlling the outcome of APP proteolytic processing and the generation of amyloid β (Aβ) peptides, the key pathogenic factor of Alzheimer's disease (AD). This study provides the first evidence to our knowledge that a G protein-coupled receptor, namely, the $\alpha_{2A}$ adrenergic receptor, modulates APP endocytic sorting and promotes Aβ generation through disrupting APP interaction with a Vps10 family protein, sorting-related receptor with A repeat. Significantly, blockade of $\alpha_{2A}$AR by a clinical antagonist reduces AD-related pathology and ameliorates cognitive deficits in an AD transgenic model, suggesting repurposing clinical $\alpha_2$AR antagonists as a new direction for AD treatment.**

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protein cleaving enzyme (BACE) 1. The initial cleavage of APP by BACE1 is the rate-limiting factor of Aβ generation (22, 23). Furthermore, blockade of $\alpha_{2A}$AR by a clinical antagonist reduces AD-related pathology and rescues cognitive deficits in an AD transgenic model, suggesting that repurposing clinical $\alpha_2$AR antagonists would be a novel effective strategy for AD treatment.

## Results

**Genetic Deficiency of the $\alpha_{2A}$AR Lessens AD-Associated Neuropathology in Vivo in an AD Mouse Model.** We first addressed whether the $\alpha_{2A}$AR plays a role in AD pathogenesis using a genetic approach. We crossed the *Adra2a*$^{-/-}$ line with the *APP/PS1* transgenic line to generate *APP/PS1,Adra2a*$^{+/+}$, *APP/PS1,Adra2a*$^{+/-}$, and *APP/PS1,Adra2a*$^{-/-}$ mice and measured the levels of Aβ peptides in the brain. ELISA analysis revealed a reduction in both Aβ40 (Fig. 1*A*) and Aβ42 (Fig. 1*B*) content in the cerebrum of 6-mo-old *APP/PS1,Adra2a*$^{-/-}$ mice compared with age- and sex-matched *APP/PS1,Adra2a*$^{+/+}$ and *APP/PS1,Adra2a*$^{+/-}$ mice. In addition, in all *APP/PS1,Adra2a*$^{-/-}$ mice examined at 6 mo of age, the cerebral Aβ deposits were significantly decreased compared with in sex-matched *APP/PS1,Adra2a*$^{+/+}$ and *APP/PS1, Adra2a*$^{+/-}$ mice at the same age (Fig. 1 *C* and *D*). These data suggest that the endogenous $\alpha_{2A}$AR activity enhances brain amyloidosis in *APP/PS1* mice. Furthermore, the number of active microglia (Fig. 1*E* and *SI Appendix*, Fig. S1) and astrocytes (*SI Appendix*, Fig. S2) in the brain of *APP/PS1,Adra2a*$^{-/-}$ mice was greatly decreased, suggesting less neuroinflammation in these mice. Taken together, our data demonstrate a critical role of the $\alpha_{2A}$AR in promoting AD-associated neuropathology.

**Activation of the $\alpha_{2A}$AR Enhances Production of Aβ Peptides in Neurons and Exacerbates Aβ Pathology in the Brain.** We next determined whether $\alpha_{2A}$AR activation enhances production of Aβ peptides in neurons. We treated primary cortical neurons with the endogenous ligand, norepinephrine (NE), in the presence of propranolol to block βARs and with prazosin to block $\alpha_1$, $\alpha_{2B}$, and $\alpha_{2C}$ARs, thus specifically activating the $\alpha_{2A}$AR. NE treatment significantly increased production of both Aβ40 (Fig. 2*A*) and Aβ42 peptides (*SI Appendix*, Fig. S3*A*). Similarly, NE treatment significantly enhanced Aβ40 generation in neuro-2a (N2a) cells expressing human APP (hAPP) (*SI Appendix*, Fig. S3*B*). Treatment with an $\alpha_2$AR-selective agonist, clonidine, which inhibited cAMP production through the $\alpha_{2A}$AR subtype in cortical neurons (*SI Appendix*, Fig. S4*A*), also increased Aβ generation (Fig. 2*B* and *SI Appendix*, Fig. S4*B*). This effect was blocked by an $\alpha_2$AR antagonist, idazoxan, and an $\alpha_{2A}$AR subtype-selective antagonist, BRL44408 (Fig. 2*B*), further demonstrating the role of the $\alpha_{2A}$ subtype receptor in promoting Aβ generation.

To determine the effect of $\alpha_{2A}$AR activation on Aβ generation in vivo, we treated C57BL/6 mice with saline or clonidine for 8 wk and examined the levels of endogenous Aβ peptides in the brain. Both Aβ40 and Aβ42 (Fig. 2*C*) levels in the cerebrum were significantly increased in mice treated with clonidine compared with age- and sex-matched mice treated with saline. We further examined the effect of $\alpha_{2A}$AR on Aβ deposition in *APP/PS1* mice. Clonidine treatment for 8 wk (starting from 4 mo of age) significantly increased Aβ load in both hippocampus and cortex of *APP/PS1* mice compared with sex-matched saline-treated controls (Fig. 2 *D* and *E*). Taken together, our in vitro and in vivo studies strongly suggest that activation of the $\alpha_{2A}$AR promotes Aβ production and exacerbates amyloid pathology.

**$\alpha_{2A}$AR Activation Disrupts APP Colocalization and Interaction with SorLA in a G Protein-Dependent Manner.** Given the important role of SorLA in controlling APP processing, we sought to address whether $\alpha_{2A}$AR signaling affects SorLA expression and/or its colocalization and interaction with APP. We found that the protein level of SorLA was not altered by $\alpha_{2A}$AR activation, nor was the full-length APP level (*SI Appendix*, Fig. S5). We then examined APP-SorLA colocalization in cells expressing hAPP

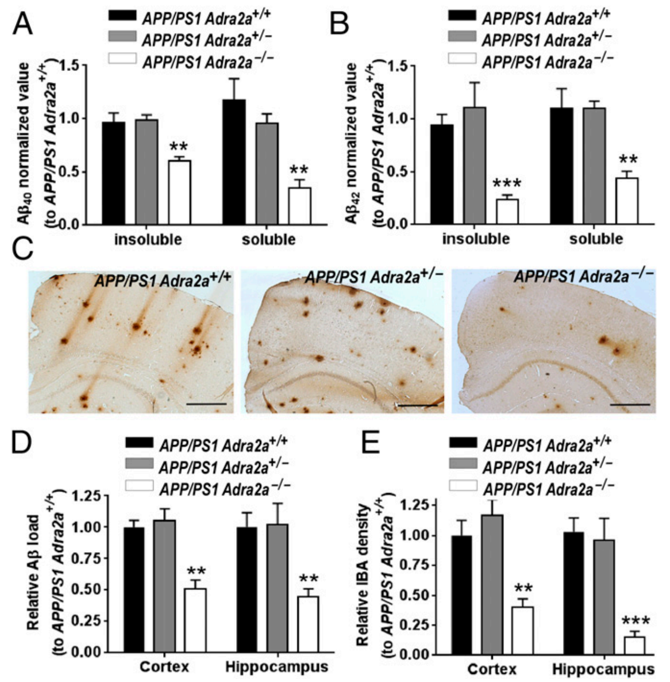


**Fig. 1.** Genetic deficiency of $\alpha_{2A}$AR reduces amyloid pathology in *APP/PS1* mice. The levels of Aβ40 (*A*) and Ab42 (*B*) in carbonate-soluble and carbonate-insoluble fractions of the cerebrum isolated from 6-mo-old male mice with indicated genotypes were measured by ELISA. $n = 6$ in each genotype measured in triplicates. Data (mean ± SEM) are expressed as the fold change to the Aβ concentration in *APP/PS1,Adra2a*$^{+/+}$ mice. $**P < 0.01$ and $***P < 0.001$ compared with *APP/PS1,Adra2a*$^{+/+}$ mice. (*C*) Representative images of Aβ staining of cerebral cortex isolated from 6-mo-old male mice with indicated genotypes. (Scale bar, 500 μm.) (*D*) Quantification of relative Aβ load. (*E*) Quantification of ionized calcium-binding adapter molecule 1 density (indicating active microglia). Data (mean ± SEM) are expressed as the fold change to the level in *APP/PS1,Adra2a*$^{+/+}$ mice. $n = 11$ for *APP/PS1, Adra2a*$^{+/+}$ and *APP/PS1,Adra2a*$^{+/-}$ mice; $n = 8$ for *APP/PS1,Adra2a*$^{-/-}$ mice. $**P < 0.01$ and $***P < 0.001$ compared with *APP/PS1,Adra2a*$^{+/+}$ mice.

and Myc-SorLA. In vehicle-treated cells, apparent colocalization between APP and SorLA was observed (Fig. 3*A*). However, the level of colocalization between these two proteins was significantly reduced after clonidine treatment (Fig. 3 *A* and *B*), suggesting that activation of the $\alpha_{2A}$AR disrupts colocalization of APP with SorLA.

We examined APP-SorLA interaction in these cells by coimmunoprecipitation (IP) assays. In cells treated with clonidine, the amount of Myc-SorLA coimmunoisolated with hAPP was significantly reduced compared with that in vehicle-treated cells (Fig. 3 *C* and *D*). However, when cells were pretreated with pertussis toxin (a $G_{i/o}$ protein blocker) or expressing a mutant $\alpha_{2A}$AR that does not couple to $G_{i/o}$ proteins (24), clonidine failed to alter APP interaction with SorLA (Fig. 3*E*). These data suggest that activation of the $\alpha_{2A}$AR disrupts APP-SorLA interaction in a G protein-dependent manner. Furthermore, clonidine treatment markedly decreased the amount of endogenous SorLA coimmunoisolated with APP in the brain (*SI Appendix*, Fig. S6*A*), suggesting that $\alpha_{2A}$AR activation disrupts APP-SorLA interaction in vivo.

When examining the level of APP in the SorLA IP complex from both cell and brain lysates, we found that clonidine treatment caused a marked reduction in the amount of mature APP coisolated with SorLA, whereas it had a marginal effect on the amount of immature APP in the SorLA IP complex compared with vehicle treatment (Fig. 3*F* and *SI Appendix*, Fig. S6*B*). These data suggest that $\alpha_{2A}$AR activation primarily disrupts SorLA interaction with the mature forms of APP. In the IP assays, two

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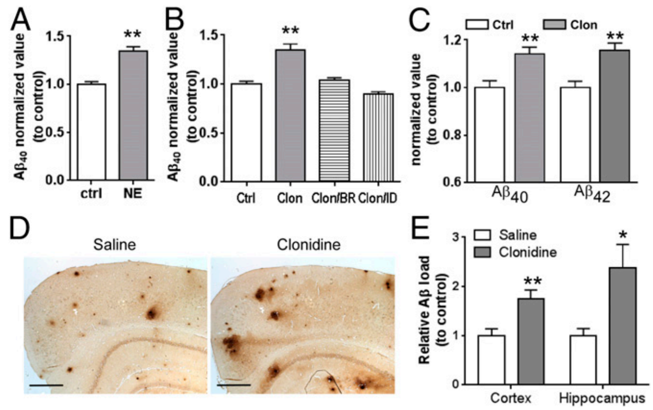


**Fig. 2.** Activation of the $\alpha_{2A}$AR enhances production of Aβ peptides and exacerbates amyloid pathology. (*A*) Cortical neurons were treated with 10μM NE (plus 1 μM propranolol and 1 μM prazosin) or vehicle for 48 h. The level of Aβ40 in culture medium was measured by ELISA. Data (mean ± SEM) are expressed as the fold change to control. $n = 9$ for each group. $^{**}P < 0.01$, NE versus control. (*B*) The level of Aβ40 secreted from cortical neurons with indicated treatment. The concentration of each drug was 1 μM. BR, BRL44408; Clon, clonidine; ID, idazoxan. Data (mean ± SEM) are expressed as the fold change to vehicle control. $n$ = 6–8 for each group. $^{**}P < 0.01$ compared with vehicle control. (*C*) The levels of endogenous Aβ40 and Aβ42 in the cortex of C57BL/6 mice treated with saline or clonidine. Data (mean ± SEM) are expressed as the fold change to the Aβ concentration in saline-treated mice. $n$ = 10–12 in each group. $^{**}P < 0.01$, clonidine versus control. (*D*) Representative images of Aβ staining of cerebral cortex isolated from *APP/PS1* mice with indicated treatment. (Scale bar, 500 μm.) (*E*) Quantification of relative Aβ load in *APP/PS1* mice with indicated treatments. Data (mean ± SEM) are expressed as the fold change to saline control. $n = 8$ for each treatment group. $^{*}P < 0.05$; $^{**}P < 0.01$, clonidine versus control.

irrelevant membrane proteins expressed in the brain, adenosine A1 receptor and Semaphorin 6D, could not be detected in the SorLA IP complex (*SI Appendix*, Fig. S7), demonstrating the specificity of APP-SorLA interaction.

**$\alpha_{2A}$AR Activation Reduces APP Localization to the Golgi and Increases Its Colocalization with BACE1 in Endosomes.** Because SorLA mediates retrograde sorting of APP to the Golgi and retains APP there, we predicted that the decrease in APP-SorLA interaction induced by $\alpha_{2A}$AR signaling would lead to reduced localization of APP in the Golgi. Indeed, in N2a cells treated with clonidine, colocalization of the endogenous APP with a Golgi marker, Golgi 58K protein (Golgi58K), was dramatically decreased compared with that in vehicle-treated control cells (Fig. 4 *A* and *B*). Concurrent with the reduction of APP localization in the Golgi, we observed a significant increase in colocalization of APP with both an early endosomal marker, early endosome antigen 1 (EEA1) (Fig. 4 *C* and *D*), and a recycling endosomal marker, Rab GTPase 11 (Rab11) (*SI Appendix*, Fig. S8 *A* and *B*), in clonidine-treated cells compared with in control cells. Furthermore, in primary cortical neurons, clonidine treatment led to a significant decrease in colocalization of endogenous APP with Golgi58K (*SI Appendix*, Fig. S8 *A* and *B*) and a simultaneous increase in colocalization of APP with EEA1 (*SI Appendix*, Fig. S9 *C* and *D*). These data suggest that activation of the $\alpha_{2A}$AR drives APP exit from the Golgi to localize in endosomes, a phenotype that is consistent with diminished SorLA regulation of APP sorting.

To confirm that distribution of the full-length mature form of APP in different organelles is altered after $\alpha_{2A}$AR activation, we performed subcellular fractionation of cells treated with or without clonidine. BACE1 was mainly distributed in endosomal fractions in both vehicle- and clonidine-treated (Fig. 5 *A* and *B* and *SI Appendix*, Fig. S10) cells. However, distribution of the mature APP appeared to be shifted from Golgi-enriched fractions to endosomal fractions after clonidine treatment (comparing Fig. 5*B* with Fig. 5*A*), but such treatment did not change the overall expression of full-length APP and BACE1 (Fig. 5*C*). The input levels of APP, BACE1, and different organelle markers are nearly identical between samples treated with vehicle and samples treated with clonidine (Fig. 5*C*). Quantitation of the percentage of mature APP distributed in different fractions revealed a significant increase of mature APP in the endosomal and BACE1-enriched fraction (fraction 6) in clonidine-treated cells compared with in control cells (Fig. 5*D*). In contrast, the percentage distribution of mature APP in the Golgi-enriched fraction (fraction 8) is significantly decreased by clonidine treatment (Fig. 5*E*).

We further examined whether $\alpha_{2A}$AR activation promotes colocalization and interaction of APP with BACE1. In cells treated with clonidine, colocalization of APP and BACE1 was significantly enhanced compared with that in control cells (Fig. 5 *F* and *G*). Moreover, clonidine treatment markedly increased the amount of BACE1 coimmunoisolated with APP compared with vehicle treatment both in cultured cells and in mouse brain

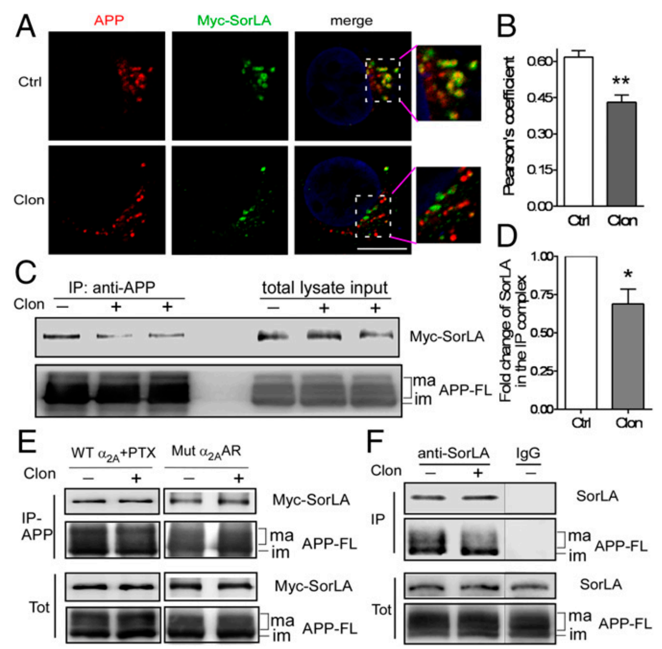


**Fig. 3.** $\alpha_{2A}$AR signaling disrupts APP-SorLA colocalization and interaction. (*A*) HEK293 cells expressing $\alpha_{2A}$AR, Myc-SorLA, and hAPP were treated with vehicle or 1 μM clonidine for 15 min. Representative images showing localization of hAPP (by Y188 antibody) and Myc-SorLA. (Scale bar, 10 μm.) (*B*) Quantification of colocalization between APP and SorLA. Data are mean ± SEM. $n$ = 17–18 cells in each group. $^{**}P < 0.01$ compared with vehicle-treated cells. (*C*) After vehicle or clonidine treatment, cell lysates were subjected to IP assays using Y188 antibody or preimmune IgG. Neither APP nor Myc-SorLA could be isolated by preimmune IgG. The input represents 1/20 of the total lysate used for each IP. APP-FL, full-length APP; im, immature form; ma, mature forms. (*D*) Quantification of the amount of SorLA in the IP complex with hAPP. Data (mean ± SEM) are expressed as fold change to vehicle control (defined as 1.0). $n = 5$ in each group. $^{*}P < 0.05$, clonidine versus control. (*E*, *Left*) Cells expressing $\alpha_{2A}$AR, Myc-SorLA, and hAPP were pretreated with pertussis toxin (PTX, a $G_{i/o}$ protein blocker, 200 ng/mL) overnight and then treated with vehicle or clonidine. (*Right*) Cells expressing a G protein-coupling deficient $\alpha_{2A}$AR, Myc-SorLA, and hAPP were treated with vehicle or clonidine. In these cells, clonidine treatment failed to alter the APP-SorLA interaction. Representative blots from three independent experiments are shown. (*F*) Interaction of endogenous APP and SorLA in mouse brain. Mice were treated with saline or clonidine for 30 min and lysates of cerebral cortex were subjected to IP assays using a SorLA antibody or preimmune IgG. Representative blots from three independent experiments were shown.

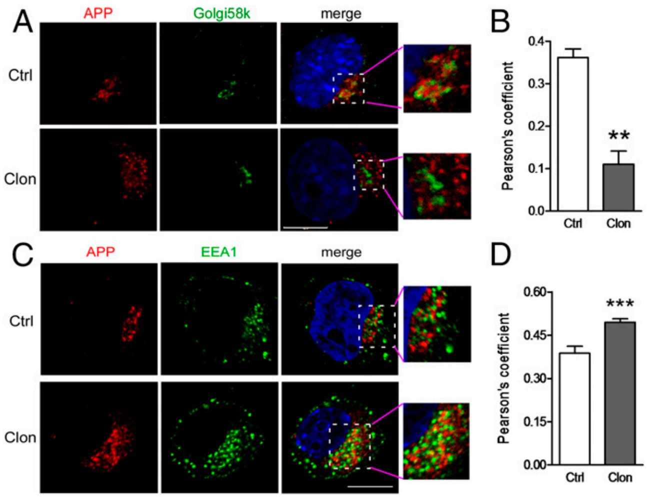


**Fig. 4.** Activation of the $\alpha_{2A}$AR reduces APP localization in Golgi and enhances its localization in endosomes. N2a cells expressing the $\alpha_{2A}$AR were treated with vehicle or 1 μM clonidine for 15 min. Representative images showing localization of the endogenous APP (by Y188 antibody) together with Golgi58K (*A*) and EEA1 (*C*). (Scale bar, 10 μm.) Colocalization was quantified by Pearson's correlation coefficient (*B* and *D*). $n$ = 13–18 cells for each group. $^{**}P < 0.01$; $^{***}P < 0.001$, clonidine versus vehicle.

We treated *APP*/*PS1* mice and sex-matched nontransgenic (nTg) littermates with a clinically used $\alpha_2$AR antagonist, idazoxan, starting at 10 wk of age, a time before Aβ deposition can be detected in the brain of *APP*/*PS1* mice. Idazoxan is highly selective and potent for blocking $\alpha_2$ARs (25) and has been used to treat mood disorders (26). After 10 wk of saline (control) or idazoxan treatment, mice were untreated for a week to allow drug washout and were then evaluated in behavioral tests for 2 wk before being killed and analyzed for pathological changes. We found that the levels of human Aβ peptides, especially Aβ42, were dramatically reduced in the cerebrum of *APP*/*PS1* mice with idazoxan treatment compared with those of mice with saline treatment (Fig. 7 *A* and *B*). Strikingly, idazoxan treatment also significantly reduced endogenous Aβ40 and Aβ42 levels in the cortex of nTg mice compared with saline treatment (*SI Appendix*, Fig. S13). In addition, in the idazoxan-treated *APP*/*PS1* mice, Aβ deposition in both hippocampus and cortex was significantly decreased compared with saline-treated controls (Fig. 7 *C* and *D*). These

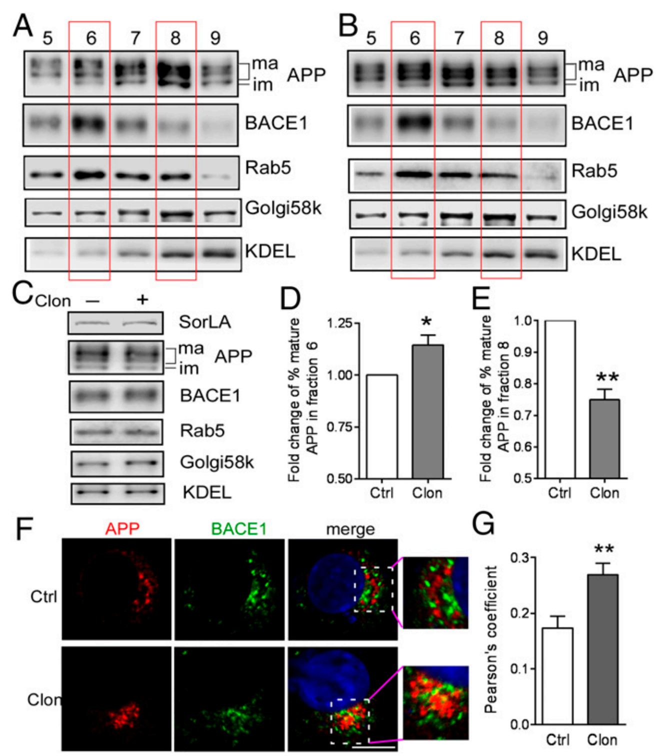


**Fig. 5.** $\alpha_{2A}$AR activation alters subcellular localization of APP and increases its colocalization with BACE1. (*A*–*E*) N2a cells expressing $\alpha_{2A}$AR were treated with vehicle (*A*) or clonidine (*B*), and cell lysates were subject to density gradient fractionation. Representative Western blots showing endogenous APP, BACE1, Rab GTPase 5 (Rab5), Golgi58K, and a KDEL (Lys-Asp-Glu-Leu) motif containing protein in fractions 5–9. The full blots with all fractions are provided in *SI Appendix*, Fig. S10. The intensity of mature APP in each fraction was quantified, and the percentage of the mature APP distributed in each fraction was calculated, with the total of all fractions equal to 100%. (*C*) Input of the total lysate subjected to fractionation in *A* (−Clon) and *B* (+Clon). (*D*) Fold change of the percentage of the mature APP in fraction 6 (where BACE1 and Rab5 peak) in clonidine-treated cells compared with control cells (arbitrarily defined as 1.0). (*E*) Fold change of the percentage of mature APP in fraction 8 (where Golgi58K peaks) in clonidine-treated cells over that in control cells (defined as 1.0). Data are mean ± SEM. $n$ = 6 for each group. $^{*}P < 0.05$; $^{**}P < 0.01$ compared with control. (*F*) N2a cells expressing $\alpha_{2A}$AR and BACE1-CFP were treated with vehicle or clonidine. The low level of BACE1-CFP expression was detected by an anti-GFP antibody. Representative images showing localization of the endogenous APP and BACE1-CFP. (*G*) Colocalization between APP and BACE1 was quantified. Data are mean ± SEM. $n$ = 14–18 cells in each group. $^{**}P < 0.01$ clonidine versus vehicle.

(*SI Appendix*, Fig. S11). Collectively, our data suggest that by diminishing the APP-SorLA interaction, $\alpha_{2A}$AR signaling promotes APP sorting to endosomal compartments, where it is colocalized and interacts with BACE1.

**Activation of the $\alpha_{2A}$AR Promotes Amyloidogenic Processing of APP in Vitro and in Vivo.** On the basis of the data shown here, we predicted that APP cleavage by BACE1 would be increased in cells after $\alpha_{2A}$AR stimulation. To test this prediction, we used cells expressing human APP carrying the Swedish mutation (hAPPsw), which produced an excess amount of the C-terminal fragment derived from BACE1 cleavage (C99) that can be readily detected by Western blot analysis. Indeed, clonidine treatment dramatically enhanced the level of C99 (Fig. 6 *A* and *B*), while having no obvious effect on the level of C83, the C-terminal fragment derived from α secretase cleavage (*SI Appendix*, Fig. S12*A*). Consistently, the amount of secreted APP ectodomain derived from BACE1 cleavage (sAPPβ; Fig. 6 *C* and *D*), but not that derived from α secretase (sAPPα; *SI Appendix*, Fig. S12*B*), was significantly enhanced by clonidine treatment. Furthermore, chronic treatment with clonidine led to a marked increase in accumulation of C99 (Fig. 6 *E* and *F*) and sAPPβ (Fig. 6 *G* and *H*), but not C83 and sAPPα (*SI Appendix*, Fig. S12 *C* and *D*), in the cerebral cortex of *APP*/*PS1* mice, suggesting that $\alpha_{2A}$AR activation promotes BACE1 cleavage of APP in vivo.

$\alpha_{2A}$AR-promoted Aβ production may also result from enhancement of γ secretase cleavage after agonist treatment. To investigate this possibility, we examined Aβ production in cells expressing $\alpha_{2A}$AR and the C99 fragment of hAPP. Unlike in cells expressing the full-length hAPP (*SI Appendix*, Fig. S3*B*), neither NE nor clonidine had an effect on Aβ production in cells expressing C99 (*SI Appendix*, Fig. S12*E*). These data suggest that activation of the $\alpha_{2A}$AR does not affect γ secretase cleavage of APP. Taken together, our data suggest that activation of the $\alpha_{2A}$AR enhances the initial processing of APP by BACE1, leading to an increase in Aβ generation.

**Pharmacologic Blockade of the $\alpha_{2A}$AR at the Early Disease Stage Reduces Amyloid Pathology and Rescues Cognitive Deficits in *APP/PS1* Mice.** To directly explore the therapeutic potential of the $\alpha_{2A}$AR, we examined whether pharmacological blockade of this receptor delays the onset of AD-related pathology in AD transgenic mice.

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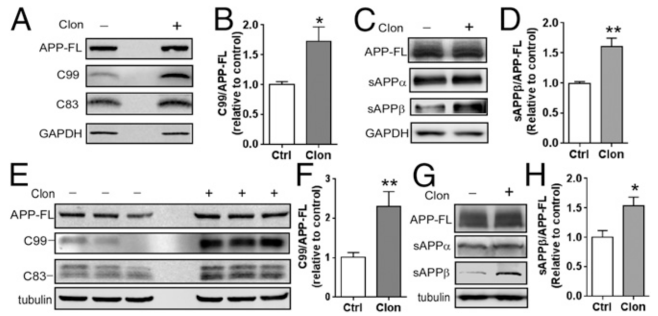


**Fig. 6.** $\alpha_{2A}$AR activation promotes amyloidogenic processing of APP. (*A–D*) N2a cells expressing $\alpha_{2A}$AR and hAPPsw were treated with clonidine or vehicle for 36 h. Representative blots of APP-FL (by Y188 antibody), C99 (by 6E10 antibody), and C83 (by Y188 antibody) separated by a tricine gel are shown in *A*. Representative blots of APP-FL, sAPPβ (by sAPPβ antibody), and sAPPα (by 6E10 antibody) separated by a glycine gel are shown in *C*. Quantitation of C99 (*B*) and sAPPβ (*D*) as a ratio over APP-FL was measured. Data represent mean ± SEM. $n = 5$–6 for each group. $*P < 0.05$; $**P < 0.01$, clonidine versus control. (*E–H*) *APP/PS1* mice treated with clonidine or saline for 8 wk. Representative blots of APP-FL, C99, and C83 in cortical lysates separated by a tricine gel are shown in *E*. Representative blots of APP-FL, sAPPα, and sAPPβ separated by a glycine gel are shown in *G*. Quantitation of C99 (*F*) and sAPPβ (*H*) as a ratio over APP-FL was measured. $n = 5$–6 for each treatment. $*P < 0.05$, $**P < 0.01$ clonidine versus control.

data suggest that $\alpha_2$AR antagonist treatment is effective in reducing Aβ generation and AD-related pathology.

We assessed basic and cognitive behaviors of *APP/PS1* mice and their age- and sex-matched nTg littermates after saline or idazoxan treatment. We did not observe any significant differences in baseline activity (*SI Appendix*, Fig. S14) or anxiety levels (*SI Appendix*, Fig. S15) between different genotypes or between different treatments. In the novel object recognition paradigm, *APP/PS1* mice treated with saline exhibited a significant deficit in recognition memory compared with saline-treated nTg littermates (Fig. 7*E*). In contrast, the performance of *APP/PS1* mice treated with idazoxan was comparable to that of their nTg littermates in this paradigm (Fig. 7*E*), suggesting that idazoxan treatment enhanced cognitive function in these mice. We further evaluated *APP/PS1* and nTg mice in the Morris water maze task. Saline-treated *APP/PS1* mice showed greatly prolonged escape latency (Fig. 7*F*) and travel distance (*SI Appendix*, Fig. S16*A*) compared with their nTg littermates receiving the same treatment. Idazoxan treatment fully rescued this spatial learning deficit in *APP/PS1* mice (Fig. 7*F* and *SI Appendix*, Fig. S16*A*). In addition, idazoxan rescued the spatial memory deficit in *APP/PS1* mice, as revealed by increased time spent in the target quadrant by *APP/PS1* mice treated with idazoxan compared with *APP/PS1* mice treated with saline (*SI Appendix*, Fig. S16 *B* and *C*). These data strongly suggest that $\alpha_2$AR antagonist treatment is effective in ameliorating AD-related cognitive deficits. Because we incorporated a 1-wk washout period before behavioral assessment, the effect of idazoxan on behavior is unlikely to be attributed to an acute symptomatic response to this drug but, rather, is a result of underlying changes in pathology caused by the chronic treatment. Taken together, our preclinical studies using a clinical $\alpha_2$AR antagonist strongly support pharmacological blockade of $\alpha_2$AR as a novel therapeutic strategy for AD treatment.

## Discussion

SorLA-dependent endocytic sorting of APP has emerged as a central regulatory mechanism of APP proteolytic processing and Aβ production (8). Our current study reveals for the first time to our knowledge that this process can be regulated by a GPCR; namely, the $\alpha_{2A}$AR, which disrupts colocalization and interaction between SorLA and the mature APP through G protein-dependent signaling and thus diminishes SorLA-dependent regulation of mature APP sorting. As illustrated in *SI Appendix*, Fig. S17, SorLA can govern APP sorting in multiple ways along the APP trafficking route. SorLA can retain APP in the Golgi, thus impeding its sorting to plasma membrane and endocytic organelles (14). In endosomes, SorLA can mediate APP retrograde sorting back to the Golgi through interacting with the retromer complex (12, 13). Consistent with diminished SorLA regulation of APP sorting, activation of the $\alpha_{2A}$AR promotes APP to exit the Golgi and concurrently increases its localization in endosomes and convergence with BACE1. As a result, more APP is processed by BACE1, and more Aβ is generated after $\alpha_{2A}$AR activation. Our current study provides the first example to our knowledge that a GPCR promotes APP amyloidogenic processing through modulating its endocytic sorting by Vps10 family receptors. A few GPCRs, including $\beta_2$AR (27) and GPR3 (28), have been reported to promote Aβ generation by enhancing γ secretase activity. However, $\alpha_{2A}$AR activation has no effect on γ secretase cleavage of the C99 fragment of APP. Modulation of APP-SorLA interaction by $\alpha_{2A}$AR represents a novel mechanism by which extracellular stimuli regulate APP processing and Aβ production.

We coisolated both mature and immature forms of APP in a complex with SorLA from cell and mouse brain lysates, suggesting that association of these two proteins can occur in the

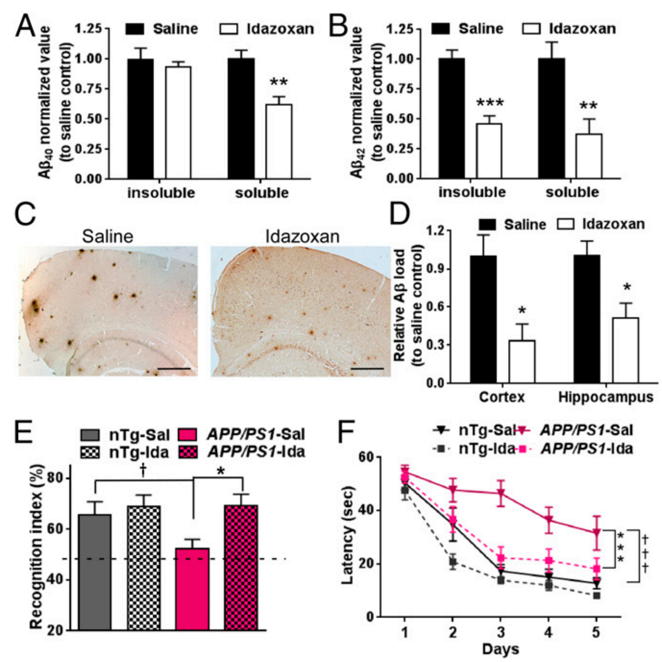


**Fig. 7.** Idazoxan treatment reduces Aβ production and deposition and ameliorates cognitive deficits in *APP/PS1* mice. The levels of Aβ40 (*A*) and Aβ42 (*B*) in carbonate-soluble and carbonate-insoluble fractions of the cerebrum isolated from *APP/PS1* mice with indicated treatment were measured by ELISA. $n = 6$ in each group. Data (mean ± SEM) are expressed as the fold change to saline treatment. $*P < 0.05$; $**P < 0.01$; $***P < 0.001$, idazoxan versus saline. (*C*) Representative images of Aβ staining in cerebral cortex. (Scale bar, 500 μm.) (*D*) Quantification of the Aβ load in different brain regions. Data (mean ± SEM) are the fold change to saline treatment. $n = 6$ in each group. $*P < 0.05$, idazoxan versus saline. (*E*) Performance of nontransgenic (nTg) and *APP/PS1* mice treated with saline or clonidine in novel object recognition tests. The dashed line indicates the 50% level of recognition index. Data represent mean ± SEM. $n = 11$ for nTg + saline (Sal); $n = 9$ for nTg + idazoxan (Ida); $n = 11$ for *APP/PS1* + saline; $n = 8$ for *APP/PS1* + idazoxan. $*P < 0.05$, saline-treated versus clonidine-treated *APP/PS1*. †$P < 0.05$, saline-treated *APP/PS1* versus nTg. (*F*) Measurement of escape latency on each day in Morris water maze tests. Data represent mean ± SEM $n = 11$ for nTg + saline and *APP/PS1* + saline; $n = 9$ for nTg + idazoxan; $n = 8$ for *APP/PS1* + idazoxan. $*P < 0.05$; $***P < 0.001$, saline-treated versus idazoxan-treated *APP/PS1* mice. †$P < 0.05$; †††$P < 0.001$, saline-treated *APP/PS1* versus nTg.

early secretary pathway. Interestingly, $\alpha_{2A}$AR activation primarily disrupted SorLA interaction with the mature forms of APP but only had a marginal effect on its interaction with immature APP. This result is consistent with our observation that $\alpha_{2A}$AR signaling does not alter APP maturation but, rather, regulates mature APP distribution between Golgi and endosomes. These data provide additional support to the specific role for $\alpha_{2A}$AR in modulation of SorLA-dependent mature APP trafficking. $\alpha_{2A}$AR-induced modulation of APP-SorLA interaction requires activation of the $G_{i/o}$ subfamily of heterotrimeric G proteins, which may regulate multiple downstream signaling effectors to modify APP and/or SorLA. How the $\alpha_{2A}$AR-induced G protein signaling cascade regulates the APP-SorLA interaction warrants further investigation.

Familial AD with mutations in genes encoding APP or PS1 (a subunit of γ secretase) accounts for less than 10% of AD. In contrast, late-onset sporadic AD likely involves multiple genetic and environmental risk factors that lead to disruption of Aβ homeostasis. The NA system plays a pivotal role in supporting interactions with and responses to environmental stimuli, and modulates cognitive activities in the cortex, including attention, perception, and memory retrieval. Profound NA degeneration and adaptation occur consistently in patients at the early stage of AD, suggesting a potential connection between alteration of the NA system and AD. The potential involvement of NA dysfunction and AD is much more complicated than a simple loss of NE input to cerebral cortex. In fact, normal, or even elevated, NE levels have been found in patients with advanced AD, likely as a result of compensatory changes in the remaining locus coeruleus neurons, such as an increase in sprouting and NE synthesis and a decrease in NE transporter expression (29). Our current study suggests that activation of the $\alpha_{2A}$AR by NE promotes Aβ generation and exacerbates AD-related pathology. Blockade of $\alpha_{2A}$AR activity would reduce the detrimental effects of NE on AD-related pathology through this receptor subtype and would further boost NE, given that the $\alpha_{2A}$AR is also an autoreceptor, to improve cognition. Increased $\alpha_{2A}$AR density and/or activity have been associated with type 2 diabetes mellitus and depression (30, 31), both of which are risk factors for AD. $\alpha_{2A}$AR-promoted Aβ generation likely acts as a key contributor driving AD-related pathophysiology under these disease conditions. Consistent with a role of $\alpha_{2A}$AR in promoting AD, clonidine treatment has been reported to worsen attention and memory deficit in patients with AD (32).

To date, there are no effective treatments to prevent, cure, or even slow the progression of AD. Our studies suggest that the $\alpha_{2A}$AR is a previously unappreciated therapeutic target for AD treatment. Of particular significance, several $\alpha_2$AR antagonists, such as idazoxan (used in this study), mirtazapine, and yohimbine, have been widely used clinically for the treatment of mood disorders and sexual dysfunction, as well as for body fat loss. Redirection of these existing therapeutics to treat AD would greatly expedite clinical trials, as their safety, brain bioavailability, and pharmacokinetic/pharmacodynamic parameters have been well addressed previously, thus saving the time and cost associated with new drug development.

## Materials and Methods

Mice were housed in the Association for Assessment and Accreditation of Laboratory Animal Care-accredited Animal Resources Program facility at the University of Alabama at Birmingham. All strains of mice have been backcrossed for more than 12 generations to C57BL/6 background. Experimental details on drug treatment, behavioral assessment, and immunohistochemistry for amyloid plaques and activated astrocytes/microglia are provided in *SI Appendix, Materials and Methods*. The levels of Aβ1-42 and Aβ1-40 were determined using specific ELISA kits, as described in *SI Appendix, Materials and Methods*. Detailed information on cell culture, immunocytochemistry, immunoprecipitation, subcellular fractionation, and Western blot are provided in *SI Appendix, Materials and Methods*.

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