Inhibiting α1-adrenergic Receptor Signaling Pathway Ameliorates Alzheimer’s Disease Type Pathologies and Behavioral Deficits in an AD Mouse Model

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Research

Keywords: Alzheimer's disease, α1-adrenergic receptor, Terazosin, Amyloid-beta, Tau.

Posted Date: August 30th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-824580/v1

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Abstract

Background The role of α1 adrenergic receptors (α1-ARs) signaling pathway in the pathogenesis of Alzheimer’s disease (AD) has rarely been investigated. Clarifying pathophysiological functions of α1-ARs in the AD brain is helpful for better understanding the pathogenesis and screening novel therapeutic target of AD.

Methods This study included 2 arms of in vivo investigations: 1) 6-month-old female APPswe/PS1 mice were intravenously treated with AAV-PHP.eB-shRNA (ARs)-GFP or AAV-PHP.eB-GFP for 3 months. 2) 3-month-old female APPswe/PS1 mice were daily treated with 0.5 mg/kg terazosin or equal saline for 6 months. SH-SY5Y cell lines bearing human Amyloid precursor protein were treated with terazosin or saline for investigating possible mechanisms.

Results α1-ARs knockdown mice exhibited improved behavioral performances than control mice. α1-ARs knockdown mice had significantly lower brain amyloid burden, as reflected by soluble Aβ species, compact and total plaques, than control mice. The α1-ARs inhibitor terazosin substantially reduced Aβ deposition, attenuated downstream pathologies including Tau hyperphosphorylation, glial activation, neuronal loss, synaptic dysfunction, and rescued behavioral deficits of APPswe/PS1 mice. In vitro investigation demonstrated that α1-ARs inhibition down-regulated BACE1 expression, and promoted ser9 phosphorylation of GSK-3β, thus reduced Aβ production.

Conclusions This study indicates that inhibition of α1-ARs signaling pathway might represent a promising therapeutic strategy for AD.

Introduction

Alzheimer’s disease (AD) is the most popular form of neurodegenerative diseases among the older adults (1). AD has two major pathological hallmarks, including the intracellular senile plaques consisted of Amyloid-beta (Aβ) plaques and the intracellular neurofilament tangles (NFT) formed by hyperphosphorylated Tau (2). It is suggested that the loss of noradrenergic neurons in the locus coeruleus (LC) represents an early pathological change in the AD brain. Noradrenergic degeneration in the AD brain simultaneously leads to compensatory changes, such as modifications of neuronal anatomy, neurotransmitter, and noradrenergic receptors (3, 4), and enhanced responses to norepinephrine (NE) that likely underlie aggressive behaviors, agitation, and sleep disturbance et al. in AD patients (5).

Previous studies have well documented the role of α2- and β-adrenergic receptors in the pathogenesis of AD (6, 7). However, the pathophysiological functions of α1 adrenergic receptors (α1-ARs) in the AD brain have been rarely investigated. A panel of studies have suggested that the expression of α1-ARs is increased in the AD brain (8–10), with the pathophysiological significance unknown. In this study, we investigated the effects of pharmacological and genetic inhibition of α1-ARs on behavioral and pathological phenotypes in APP/PS1 mice.
Materials And Methods

Animals and treatment

APPswe/PS1dE9 transgenic mice (AD mice) were obtained from Jackson Laboratory and C57BL/6 wild-type (Wt) mice were obtained from the animal center of Daping hospital, Third Military Medical University. 3-month-old AD mice were daily treated with 0.5 mg/kg terazosin or equal saline intraperitoneally from 3 months old to 9 months old (n = 8 per group). 6-month-old AD mice were treated with AAV-PHP.eB-shRNA (α1-ARs)-GFP for 3 months. Control AD mice were treated with a same amount of AAV-PHP.eB-GFP. At 9 months of age, mice were subjected to behavioral tests and were sacrificed for biochemical and histological experiments. All animal experimental protocols were approved by Third Military Medical University Animal Welfare Committee and performed according to the guidelines of laboratory animal care and use.

Behaviour tests

After treatment, mice were assessed for behavioral analyses using the Morris water maze (MWM), Y-maze, elevated plus maze and open-field tests. All behavioral performances were tracked and analyzed with a computer tracking system (ANY-maze, Stoelting, Wood Dale, IL, USA).

In brief, the MWM test consisted of three platform trials per day for 5 consecutive days, followed by a probe trial. In the platform trial, the distance of the path taken and the escape latency were measured to test spatial learning ability. In the probe trial, the time spent in each quadrant and the number of annulus crossings were recorded to assess memory consolidation.

Y maze test with spontaneous alternation performance was conducted according to a previous study (11). Each mouse was placed in the center of the symmetrical Y maze and was allowed to explore freely through the maze for 5 minutes. The sequence and total number of arms entered were recorded. Percentage of alternation was determined as follows: number of trials containing entries into all three arms/maximum possible alternations × 100. The maximum possible alterations = total number of arms entered − 2.

In the novel arm exploration, one arm (defined as the novel arm) was closed. After freely exploration for 5 min and 25 min interval, mice were resubjected to apparatus for the retrieval trial, during which mice were allowed to explore all arms freely for 5 min. The number of entries and time spent in the closed arm (novel arm) were analyzed.

The open field test was conducted to measure the spontaneous locomotor activity. The apparatus was made of four transparent Plexiglass walls (60cm high) and a gray 40cm x 40cm floor. Mice were subjected to apparatus for freely exploring for 5 min. The total distance travelled and average speed were analyzed.
The Elevated Plus Maze test is conducted to assess anxiety-related behavior. The EPM apparatus consists of a "+"-shaped maze elevated above the floor with two oppositely positioned closed arms, two oppositely positioned open arms, and a center area. In brief, mice were subjected into the center of apparatus and allowed for freely exploring for 5 min. The number of entries into and time spent in the open arm were analyzed.

**Brain sampling**

All experimental mice were sacrificed at 9-month of age. After anesthetized, mice were intracardial perfusion with 0.1% NaNO₂ in 0.9% saline, the right brain hemisphere was dissected and fixed with 4% paraformaldehyde (PFA). Left brain hemispheres were snap-frozen by liquid nitrogen, ground into a powder, and stored at -80°C for biochemical analysis.

**Western blot**

All western blot experiments were performed according to our previous study (12). Brain protein mixed with 5x loading buffer were resolved using SDS-PAGE (4–20% acrylamide) at 90 V for 30 min and 120 V for 80 min, and then transferred onto nitrocellulose filter membranes at 250 mA for another 50 min. After blocked with 5% fat-free milk, membranes were immunoblottedted with the following primary antibodies: anti-6E10 (Biolegend), anti-APP-C-terminal (Millipore), anti-β-secretase 1 (BACE1) antibody (Abcam), anti-Presenilin-1 (PS1) antibody (Millipore), anti-a disintegrin and metalloproteinase 10 (ADAM10) antibody (Abcam), anti-insulin degrading enzyme (IDE) antibody (Millipore); anti-neprilysin (NEP) antibody (Millipore); anti-receptor for advanced glycation end products (RAGE) antibody (Millipore), anti-lipoprotein receptor-related protein 1 (LRP1) antibody (Abcam), anti-pT231 antibody (Signalway), anti-pS199 antibody (Invitrogen), anti-pS396 antibody (Abcam), anti-tau5 antibody (Millipore), anti-tau46 antibody (Cell signaling technology), anti-glycogen synthase kinase 3 beta (GSK3β) antibody (Abcam), anti-pS9-GSK3β antibody (Abcam), anti-synaptosomal-associated protein 25 (SNAP-25) antibody (Abcam), anti-vesicle-associated membrane protein 1 (VAMP1) antibody (Millipore), anti-postsynaptic density protein 95 (PSD95) antibody (Millipore), anti-α1-adrenergic receptor (α1-ARs) antibody (Thermo), and anti-β-actin antibody (Origene). Then membranes were washed with Tris-buffered saline containing 0.5% Tween 20 (TBST) and probed with corresponding IRDye 800 CW-conjugated secondary antibodies (1:5000) for 1 hour at room temperature and scanned using an Odyssey fluorescent scanner.

**Immunohistochemistry and immunofluorescence**

Immunohistochemistry and immunofluorescence were conducted to our previous methods (13). Serial coronal brain sections (30 µm in thickness) were cut using cryostat. Five brain sections spanning the entire brain were stained with a 6E10 antibody or Congo red (Sigma) to detect Aβ plaques in the brain (6E10 antibody for compact and diffuse Aβ; Congo red for compact Aβ). Cerebral amyloid angiopathy (CAA) was manually assessed in Congo red-stained hippocampal sections under a microscope (14). Brain sections were also incubated with anti-CD68 antibody (Abcam) and anti-glial fibrillary acidic protein (GFAP) antibody (Abcam) to assess the microgliosis and astrocytosis, respectively. Double immunofluorescence staining for NeuN and microtubule associated protein (MAP)-2 (Abcam) were
performed to detect the neuronal loss and neurite degeneration. Apoptosis of neurons in the CA3 area of hippocampus was assessed by double-immunofluorescence staining for NeuN (Abcam) and caspase-3 (Abcam).

**ELISAs**

Proteins were extracted from brain tissues in tris buffer solution (TBS), 2% sodium dodecyl sulfonate (SDS) to measure soluble and insoluble Aβ levels in brain. The levels of human Aβ42 and Aβ40 in the TBS and SDS fractions were measured using ELISA kits (Invitrogen, USA).

**Adeno-associated virus (AAV) transduction for α1-ARs interference in vivo**

100µL AAV-PHP.eB-hsyn-shRNA (α1-ARs)-GFP-pA or AAV-PHP.eB-hsyn-GFP-pA (1x10^{13}v.g/ml) was injected into tail vein of 6-month-old AD mice. The target sequence of α1-ARs is provided in AAV 5′→3′ orientation: TGCCAAGAATAAGACTCACTT, respectively. The efficiency of interfering of AAV was detected by the expression of α1-ARs using western blot.

**Cell culture and treatment**

SH-SY5Y-APP695 cells were maintained in DMEM (H) supplemented with 10% fetal bovine serum, G418 (100µg/ml), and 1% penicillin-streptomycin. SH-SY5Y-APP695 cells were seeded onto the 6-well plate or 24-well plate with a cover slide. After attachment, the cells were treated with 50µM terazosin or equal volume saline for 4 h. SH-SY5Y-APP695 cells treated with terazosin or saline were collected for immunofluorescence detection and western blot analysis.

**Statistical Analysis**

All data were expressed as the mean ± SEM and analyzed by SPSS 22.0 software (SPSS, Chicago, IL, USA). Appropriate two-tailed independent t-tests and MannWhitney U test were conducted for the comparison between two groups. One-way analysis of variance (ANOVA) followed by Tukey post hoc analysis was used for multiple comparisons. A P value < 0.05 was considered as statistically significant.

**Results**

**Knockdown of α1-ARs improved the behavioral deficits in AD mice**

The expression of α1-ARs in brain was significantly reduced 3 months after AAV-α1-ARs treatment (Supplementary Fig. 1). Mice treated with AAV-α1-ARs dispayeded better performance in both Y maze test and open-field test compared with AAV-GFP treated mice. In the Y maze test, mice treated with AAV-α1-ARs had more time spent in the novel arm and more entries into the novel arm, as well as a higher spontaneous alternation than AAV-GFP treated mice (Supplementary Fig. 2A), indicating a better performance of working memory and learning. AAV-α1-ARs treatment significantly increased the distance
travelled and the travelling speed of AD mice in the open-field test (Supplementary Fig. 2B and D). Compared with AAV-GFP group, AAV-α1-ARs treated mice seemed to display fewer anxiety-like behaviours, although no significance has been achieved (Supplementary Fig. 2C). Taken together, these findings demonstrated that the down-regulation of brain α1-ARs significantly improved behavioral deficits in AD mice.

**Knockdown of α1-ARs decreased brain Aβ burden in AD mice**

We further investigated the effect of AAV-α1-ARs treatment on Aβ burden in the brain of AD mice. 6E10 and Congo red staining were performed to detect the total Aβ plaques and compact Aβ plaques, respectively. Compared with control mice, mice treated with AAV-α1-ARs displayed a lower area fraction of 6E10-positive and Congo red-positive staining in the neocortex but not in the hippocampus (Fig. 1A). Consistently, levels both Aβ42 and Aβ40 in the TBS and SDS fractions of brain homogenates in the AAV-α1-ARs group were lower than those in the AAV-GFP group (Fig. 1B).

We next investigated the expressions of proteins involved in Aβ production, transportation and degradation. There were no differences in the expression of full-length APP and CTF-α between mice treated with AAV-α1-ARs and AAV-α1-ARs. No significance was also observed in the expression of ADAM10 and PS1 between the two groups. However, levels of BACE1 and CTF-β in AAV-α1-ARs group were significantly lower than that in AAV-GFP group (Fig. 1C and D). There were no significant differences in levels of proteins involved in Aβ transportation and degradation, including IDE, LRP1 and RAGE, between AAV-α1-ARs group and AAV-GFP group (Fig. 1E). The above findings indicated that the decreased Aβ burden after knockdown of α1-ARs might be attributed to decreased Aβ production but not increased Aβ clearance in the brain.

**Knockdown of α1-ARs attenuated other AD-type pathologies in AD mice**

Mice treated with AAV-α1-ARs exhibited lower area fraction and cell density of activated astrocytes (labeled by GFAP) in both neocortex and hippocampus (Fig. 2A). Besides, positive area fraction and cell density of activated microglia (labeled by CD68) in the neocortex but not hippocampus was significantly lower in AAV-α1-ARs group than AAV-GFP group (Fig. 2A). Western blot showed that the expression of phosphorylated tau protein, including PS396 and PS199, but not PT231, in AAV-α1-ARs group was lower than that in AAV-GFP group (Fig. 2B). Compared to AAV-GFP treated mice, mice treated with AAV-α1-ARs displayed higher expression of synapse-related proteins, including SYN and VAMP1, but not SNAP25 and PSD95 (Fig. 2C). Taken together, these findings suggested that down-regulation of α1-ARs attenuated neuroinflammation, tau hyperphosphorylation and neurodegeneration in the brain of AD mice.

**Terazosin improved behavioral deficits in AD mice**
We further investigated whether terazosin, a specific α1-ARs antagonist, had similar therapeutic effects to genetic knockdown of these receptors. Saline treated AD mice displayed an impaired learning ability and spatial reference memory in comparison with Wt mice, while mice treated with terazosin presented improved spatial learning ability, as reflected by a significant reduction in the escape latency (Fig. 3A). There were no differences in the time spent in the target quadrant and annulus crossing between terazosin and saline treated AD mice (Fig. 3A). In open-field tests, Wt mice had a longer travelling distance than AD mice treated with saline, but AD mice treated with saline and terazosin had no significant difference (Fig. 3B and C). In open field test, the ratio of time in the central area to that in the peripheral area was significantly higher in the terazosin treated group and the Wt group than that in the saline-treated group (Fig. 3C), reflecting decreased anxiety-like behaviours after terazosin treatment. Furthermore, mice treated with terazosin also exhibited higher spontaneous alternation in the Y-maze test than saline-treated mice, reflecting improved spatial recognition memory after terazosin treatment (Fig. 3D).

**Terazosin treatment significantly ameliorated Aβ burden in AD mice**

Mice treated with terazosin displayed a lower area fraction of 6E10-positive plaques and plaque density in both neocortex and hippocampus. Congo red staining also showed that the area fraction of compact plaques in neocortex and hippocampus in terazosin treated group were lower than that in saline treated group (Fig. 4A), and the compact plaque density in terazosin treated group was lower than that in saline treated group in neocortex, but not in hippocampus (Fig. 4A). Mice treated with terazosin exhibited lower load of CAA, as indicated by Congo red staining, than saline treated mice (Fig. 4B). Consistently, both Aβ40 an Aβ42 concentrations in TBS and SDS fractions of brain homogenates in the terazosin group were lower than that in the saline group (Fig. 4C).

The expression of full-length APP and its metabolites, including sAPPα and CTFα was not significantly different between terazosin and saline treated group, while the levels of Aβ dimers and CTF-β in the terazosin group were significantly lower than those in saline treated group (Fig. 4D). We also found that the expression of BACE1 in terazosin treated mice was lower than that in saline treated mice (Fig. 4E). However, there were no significant differences in other APP metabolic enzymes, Aβ transport- and degradation-related proteins, between terazosin and saline group (Fig. 4E).

**Terazosin attentuated other AD-type pathologies in AD mice**

Tau 231 immunohistochemical staining and western blot showed that the levels of phosphorylation of Tau at multiple epitopes, including PS396 and PS199, in the terazosin group were significantly lower than those in the saline group (Fig. 5A and B), whereas the expression of total tau (Tau5) didn’t differ between two groups (Fig. 5B). Levels of synapse-related proteins, including SYN and SNAP25, but not PSD95, were significantly higher in mice treated with terazosin in comparison with mice treated with saline (Fig. 5C).
Compared with mice treated with saline, terazosin treated mice displayed higher area fractions of viable neurons (NeuN) and dendrites (Map-2), but lower area fractions of apoptosis-related protein (caspase-3) in the hippocampus (Fig. 5D). Additionally, mice treated with terazosin exhibited lower area fractions of activated astrocytes in both neocortex and hippocampus than saline treated mice (Fig. 5E and F). Terazosin treated group had significantly lower area fractions and cell density of activated microglia in both neocortex and hippocampus than saline treated group (Fig. 5E and F). Overall, the above findings suggested that terazosin could ameliorate neuroinflammation, tau hyperphosphorylation and neurodegeneration in brains of AD mice.

**Terazosin reduced Aβ production through inhibiting GSK3β/BACE1 signaling pathway**

To explore the mechanism underlying reduced Aβ production by inhibition of α1-ARs, SH-SY5Y-APP695 cells, which overexpress human APP, were treated with 50µM terazosin for 4 h. Western blot showed that the terazosin did not affect the expression of the full-length APP, but decreased the ratio of CTF-β to CTF-α (Fig. 6A). We also measured Aβ40 and Aβ42 levels in cell lysates and cell medium, and found that terazosin treatment decreased the levels of both Aβ40 and Aβ42 in cell lysates and cell medium (Fig. 6A). In addition, the expression of BACE1, but not LRP1 and PS1, was significantly decreased after terazosin treatment (Fig. 6B and C), indicating that the reduction of Aβ in SH-SY5Y-APP695 cells after terazosin treatment might be resulted from decreased expression of BACE1. Furthermore, we also found that terazosin increased the expression of pGSK3β, which could inhibit the activity of GSK3β and result in decreased BACE1 expression (Fig. 6D).

**Discussion**

In this study, we found that both genetical knockdown and pharmacological inhibition of α1-ARs alleviated behavioral deficits and pathological changes in an AD mouse model. Mechanistically, we suppose that inhibition of α1-ARs may exert neuroprotective effects against AD by inhibiting GSK3β/BACE1 signaling pathway, thus reducing Aβ production and subsequent neurodegeneration in the brain.

NE signaling plays a critical role in maintaining cognitive functions, but the noradrenergic system is suggested to be dysfunctional in the AD brain (3). There is profound neurodegeneration in the LC with AD progressing, but its pathological significance has not been thoroughly addressed to date. Enhancement in neuronal sprouting to different brain regions and NE production (4, 9, 15), and reduction in NE reuptake (16, 17) might compensate for LC neuronal loss to maintain NE levels in the AD brain. As a consequence of these compensatory changes, extracellular NE is suggested to be increased in the AD brain (18, 19). Furthermore, the increase in extracellular NE has been shown to be associated with the severity of AD (20, 21), with higher NE levels associating with more severe cognitive impairment. In addition, densities of α1-adrenergic receptors are suggested to be increased in the AD brain (4, 10). These findings may imply that although NE is important for maintaining cognitive functions, over-compensation of NE during the
process of AD could be detrimental. This notion is supported by studies that have shown cognitive impairment in response to stress, because under stress conditions NE release is elevated (22, 23). Therefore, inhibition of the NE signaling pathway in the AD brain might be helpful for controlling the dysregulated NE system and aid in rescuing cognitive impairment. Previous studies have shown that signaling of α2 and β2-adrenergic receptors is detrimental, and inhibition of these adrenergic receptors improves behavioral deficits and AD-type pathologies in AD mouse models (6, 7). Although activation of the α1-ARs signaling pathway improves cognitive in non-AD animals (24–26), blockade of α1-ARs by prazosin also improves the cognitive deficits of an AD transgenic mouse model (27). In AD patients, activation of α1-ARs is suggested to contribute to the agitation behaviors (26), and prazosin can alleviate such symptoms (28). These results indicate that the activation of α1-ARs by NE may contribute to agitation and aggressive behaviors, which are commonly seen in AD patients. Mice overexpressing α1-ARs had enhanced expression of genes involved in apoptosis and neurodegeneration (29), further supporting the possible pathological effects of α1-ARs in the AD brain. In accordance with this hypothesis, we found in this study that genetical and pharmacological inhibition of α1-ARs alleviated behavioral cognitive deficits and attenuated AD-type pathologies, including Aβ deposition, Tau hyperphosphorylation, neuroinflammation and neuronal degeneration et al.

In this study, we found that terazosin inhibited GSK3β pathway in vitro, as reflected by increased pGSK3β expression after terazosin treatment. This finding is consistent with a previous study which found that doxazosin, another α1-ARs antagonist, also increased the levels of pGSK3β (30). It is well-recognized that activation of GSK-3β could increase the expression and activity of BACE1, thus promoting the amyloidogenic pathway (31, 32). Indeed, we found in this study that inhibition of α1-ARs reduced BACE1 expression both in vivo and in vitro, thus reduced Aβ production. Therefore, inhibition of the α1-AR pathway might attenuate Aβ pathologies through promoting ser9 phosphorylation of GSK3β. Interestingly, it is suggested that Aβ could in return activate α1-ARs (33), therefore, α1-ARs activation and Aβ accumulation during the pathogenesis of AD may form a vicious circle, which could be disrupted by inhibition of α1-ARs. Furthermore, a study found that terazosin rescues pathologies of Parkinson's disease through enhancing glycolysis in the brain (34). Energy metabolism dysfunction and reduced ATP levels in the brain are common features of AD. Impaired glucose metabolism could significantly promote the expression of BACE1 (35). Therefore, we suppose that inhibition of α1-ARs signaling pathway may also reduce brain amyloid burden through improving energy metabolism.

In this study, both genetical and pharmacological inhibition of α1-ARs was found to reduce NFT formation and suppress Tau hyperphosphorylation at several sites. GSK3β activation also promotes Tau hyperphosphorylation, and ser9 phosphorylation of GSK3β inhibits this process (36), thus down-regulation of Tau hyperphosphorylation after inhibiting α1-ARs might be directly associated with its effect on promoting ser9 phosphorylation of GSK3β. Besides, amelioration of Aβ pathology might also contribute to reduced NFT formation and Tau hyperphosphorylation (37). Furthermore, other pathologies subsequent to Aβ or Tau, including cerebral amyloid angiopathy (CAA), overactivation of glia cells, down-regulation of synaptic proteins, dendritic damage and neuronal apoptosis et al. were also improved after
inhibiting α1-ARs signaling pathways. We suppose that inhibition of α1-ARs may act on Aβ and suppress its toxicity of triggering subsequent pathological changes.

**Conclusion**

Our findings prompt a possibility that over-compensation of the α1-ARs system in the AD brain might be detrimental, thus suitable down-regulating the expression and pharmacological inhibition of α1-ARs may be beneficial for AD. Furthermore, terazosin is a clinically used drug which has validated tolerability; thus, it may hold promise for the treatment of AD.

**Abbreviations**

Alzheimer’s disease (AD); Amyloid-beta (Aβ); intracellular neurofilament tangles (NFT); locus coeruleus (LC); norepinephrine (NE); α1 adrenergic receptors (α1-ARs); neprilysin (NEP); receptor for advanced glycation end products (RAGE); lipoprotein receptor-related protein 1 (LRP1); glycogen synthase kinase 3 beta (GSK3β); synaptosomal-associated protein 25 (SNAP-25); vesicle-associated membrane protein 1 (VAMP1); postsynaptic density protein 95 (PSD95); Tris-buffered saline containing 0.5% Tween 20 (TBST); glial fibrillary acidic protein (GFAP); microtubule associated protein (MAP)-2; sodium dodecyl sulfate (SDS); Adeno-associated virus (AAV);

**Declarations**

**Acknowledgements**

None.

**Authors’ contributions**

LYH and WYJ designed this study and drafted the manuscript. YZY, YX and WYR conducted the experiments. ZGH and TCR bred the animals. CY, SPY, and LZH conducted statistical analyses.

**Funding**

This study was supported by the National Natural Science Foundation of China (No. 81971024).

**Availability of data and materials**

All data generated or analyzed during this study are included in this article.

**Ethics approval and consent to participate**

This study was performed in accordance with the ethical review and received approval from the Institutional Animal Care and Use Committee of Daping hospital.
**Consent for publication**

All contributing authors have given their consent for the publication of this study.

**Competing interests**

The authors declare that they have no competing interests.

**References**

1. Jia L, Du Y, Chu L, Zhang Z, Li F, Lyu D, et al. Prevalence, risk factors, and management of dementia and mild cognitive impairment in adults aged 60 years or older in China: a cross-sectional study. Lancet Public Health. 2020;5(12):e661-e71.

2. Scheltens P, De Strooper B, Kivipelto M, Holstege H, Chetelat G, Teunissen CE, et al. Alzheimer's disease. Lancet. 2021.

3. Gannon M, Che P, Chen Y, Jiao K, Roberson ED, Wang Q. Noradrenergic dysfunction in Alzheimer's disease. Front Neurosci. 2015;9:220.

4. Szot P, White SS, Greenup JL, Leverenz JB, Peskind ER, Raskind MA. Compensatory changes in the noradrenergic nervous system in the locus ceruleus and hippocampus of postmortem subjects with Alzheimer's disease and dementia with Lewy bodies. J Neurosci. 2006;26(2):467-78.

5. Herrmann N, Lanctot KL, Khan LR. The role of norepinephrine in the behavioral and psychological symptoms of dementia. J Neuropsychiatry Clin Neurosci. 2004;16(3):261-76.

6. Chen Y, Peng Y, Che P, Gannon M, Liu Y, Li L, et al. alpha(2A) adrenergic receptor promotes amyloidogenesis through disrupting APP-SorLA interaction. Proc Natl Acad Sci U S A. 2014;111(48):17296-301.

7. Zhang F, Gannon M, Chen Y, Yan S, Zhang S, Feng W, et al. beta-amyloid redirects norepinephrine signaling to activate the pathogenic GSK3beta/tau cascade. Science translational medicine. 2020;12(526).

8. Sharp SI, Ballard CG, Chen CP, Francis PT. Aggressive behavior and neuroleptic medication are associated with increased number of alpha1-adrenoceptors in patients with Alzheimer disease. The American journal of geriatric psychiatry : official journal of the American Association for Geriatric Psychiatry. 2007;15(5):435-7.

9. Szot P, White SS, Greenup JL, Leverenz JB, Peskind ER, Raskind MA. Changes in adrenoreceptors in the prefrontal cortex of subjects with dementia: evidence of compensatory changes. Neuroscience. 2007;146(1):471-80.

10. Klingner M, Apelt J, Kumar A, Sorger D, Sabri O, Steinbach J, et al. Alterations in cholinergic and non-cholinergic neurotransmitter receptor densities in transgenic Tg2576 mouse brain with beta-amyloid plaque pathology. Int J Dev Neurosci. 2003;21(7):357-69.
11. Zhu H, Wang X, Wallack M, Li H, Carreras I, Dedeoglu A, et al. Intraperitoneal injection of the pancreatic peptide amylin potently reduces behavioral impairment and brain amyloid pathology in murine models of Alzheimer’s disease. Mol Psychiatry. 2015;20(2):252-62.

12. Yu Z, Yang L, Yang Y, Chen S, Sun D, Xu H, et al. Epothilone B Benefits Nigral Dopaminergic Neurons by Attenuating Microglia Activation in the 6-Hydroxydopamine Lesion Mouse Model of Parkinson’s Disease. Front Cell Neurosci. 2018;12:324.

13. Wang J, Jin WS, Bu XL, Zeng F, Huang ZL, Li WW, et al. Physiological clearance of tau in the periphery and its therapeutic potential for tauopathies. Acta Neuropathol. 2018;136(4):525-36.

14. Wilcock DM, Gordon MN, Morgan D. Quantification of cerebral amyloid angiopathy and parenchymal amyloid plaques with Congo red histochemical stain. Nat Protoc. 2006;1(3):1591-5.

15. Nelson AR, Kolasa K, McMahon LL. Noradrenergic sympathetic sprouting and cholinergic reinnervation maintains non-amyloidogenic processing of AbetaPP. J Alzheimers Dis. 2014;38(4):867-79.

16. Gulyas B, Brockschnieder D, Nag S, Pavlova E, Kasa P, Beliczai Z, et al. The norepinephrine transporter (NET) radioligand (S,S)-[18F]FMeNER-D2 shows significant decreases in NET density in the human brain in Alzheimer’s disease: a post-mortem autoradiographic study. Neurochem Int. 2010;56(6-7):789-98.

17. Tejani-Butt SM, Yang J, Zaffar H. Norepinephrine transporter sites are decreased in the locus coeruleus in Alzheimer’s disease. Brain Res. 1993;631(1):147-50.

18. Czech C, Berndt P, Busch K, Schmitz O, Wiemer J, Most V, et al. Metabolite profiling of Alzheimer’s disease cerebrospinal fluid. PLoS One. 2012;7(2):e31501.

19. Kaddurah-Daouk R, Rozen S, Matson W, Han X, Hulette CM, Burke JR, et al. Metabolomic changes in autopsy-confirmed Alzheimer’s disease. Alzheimers Dement. 2011;7(3):309-17.

20. Elrod R, Peskind ER, DiGiacomo L, Brodkin KL, Veith RC, Raskind MA. Effects of Alzheimer’s disease severity on cerebrospinal fluid norepinephrine concentration. Am J Psychiatry. 1997;154(1):25-30.

21. Oishi M, Mochizuki Y, Yoshihashi H, Takasu T, Nakano E. Laboratory examinations correlated with severity of dementia. Ann Clin Lab Sci. 1996;26(4):340-5.

22. Finlay JM, Zigmond MJ, Abercrombie ED. Increased dopamine and norepinephrine release in medial prefrontal cortex induced by acute and chronic stress: effects of diazepam. Neuroscience. 1995;64(3):619-28.

23. Goldstein LE, Rasmussen AM, Bunney BS, Roth RH. Role of the amygdala in the coordination of behavioral, neuroendocrine, and prefrontal cortical monoamine responses to psychological stress in the rat. J Neurosci. 1996;16(15):4787-98.

24. Do Monte FH, Souza RR, Wong TT, Carobrez Ade P. Systemic or intra-prelimbic cortex infusion of prazosin impairs fear memory reconsolidation. Behav Brain Res. 2013;244:137-41.

25. Doze VA, Papay RS, Goldenstein BL, Gupta MK, Collette KM, Nelson BW, et al. Long-term alpha1A-adrenergic receptor stimulation improves synaptic plasticity, cognitive function, mood, and longevity. Mol Pharmacol. 2011;80(4):747-58.
26. Nalepa I, Kreiner G, Bielawski A, Rafa-Zablocka K, Roman A. alpha1-Adrenergic receptor subtypes in the central nervous system: insights from genetically engineered mouse models. Pharmacol Rep. 2013;65(6):1489-97.

27. Katsouri L, Vizcaychipi MP, McArthur S, Harrison I, Suarez-Calvet M, Lleo A, et al. Prazosin, an alpha(1)-adrenoceptor antagonist, prevents memory deterioration in the APP23 transgenic mouse model of Alzheimer's disease. Neurobiol Aging. 2013;34(4):1105-15.

28. Wang LY, Shofer JB, Rohde K, Hart KL, Hoff DJ, McFall YH, et al. Prazosin for the treatment of behavioral symptoms in patients with Alzheimer disease with agitation and aggression. Am J Geriatr Psychiatry. 2009;17(9):744-51.

29. Yun J, Gaivin RJ, McCune DF, Boongird A, Papay RS, Ying Z, et al. Gene expression profile of neurodegeneration induced by alpha1B-adrenergic receptor overactivity: NMDA/GABAA dysregulation and apoptosis. Brain. 2003;126(Pt 12):2667-81.

30. Coelho BP, Gaelzer MM, Dos Santos Petry F, Hoppe JB, Trindade VMT, Salbego CG, et al. Dual Effect of Doxazosin: Anticancer Activity on SH-SY5Y Neuroblastoma Cells and Neuroprotection on an In Vitro Model of Alzheimer's Disease. Neuroscience. 2019;404:314-25.

31. Ly PT, Wu Y, Zou H, Wang R, Zhou W, Kinoshita A, et al. Inhibition of GSK3beta-mediated BACE1 expression reduces Alzheimer-associated phenotypes. J Clin Invest. 2013;123(1):224-35.

32. Phiel CJ, Wilson CA, Lee VM, Klein PS. GSK-3alpha regulates production of Alzheimer's disease amyloid-beta peptides. Nature. 2003;423(6938):435-9.

33. Haase N, Herse F, Spallek B, Haase H, Morano I, Qadri F, et al. Amyloid-beta peptides activate alpha1-adrenergic cardiovascular receptors. Hypertension. 2013;62(5):966-72.

34. Cai R, Zhang Y, Simmering JE, Schultz JL, Li Y, Fernandez-Carasa I, et al. Enhancing glycolysis attenuates Parkinson's disease progression in models and clinical databases. J Clin Invest. 2019;129(10):4539-49.

35. Velliquette RA, O'Connor T, Vassar R. Energy inhibition elevates beta-secretase levels and activity and is potentially amyloidogenic in APP transgenic mice: possible early events in Alzheimer's disease pathogenesis. J Neurosci. 2005;25(47):10874-83.

36. Qian W, Shi J, Yin X, Iqbal K, Grundke-Iqbal I, Gong CX, et al. PP2A regulates tau phosphorylation directly and also indirectly via activating GSK-3beta. J Alzheimers Dis. 2010;19(4):1221-9.

37. Laversenne V, Nazeeruddin S, Kallstig EC, Colin P, Voize C, Schneider BL. Anti-Abeta antibodies bound to neuritic plaques enhance microglia activity and mitigate tau pathology. Acta Neuropathol Commun. 2020;8(1):198.

**Figures**
Knockdown of α1-ARs decreased brain Aβ burden in AD mice. A. Immunostaining and quantification of Aβ plaques stained with 6E10 and Congo red in the neocortices and hippocampi of AD mice treated with AAV-GFP or AAV-α1-ARs. B. Comparison of Aβ40 and Aβ42 levels in TBS and SDS fractions of brain homogenates between the AAV-GFP and AAV-α1-ARs group. C-E. Representative western blots and quantitative analysis of the levels of APP-metabolizing and Aβ-degrading enzymes as well as Aβ-
transporting receptors brain homogenates. n=7-8 per group; * indicates P<0.05 and ** indicates P<0.01. Scale bar in A is 500 µm. Data are represented as mean ± SEMs.

Figure 2

Knockdown of α1-ARs attenuated the AD-related pathology in AD mice. A. Immunostaining of astrocytes and microglia with GFAP and CD68 in the neocortices and hippocampus of AD mice treated with AAV-GFP or AAV-α1-ARs. B. Representative western blot images and quantification of phosphorylated Tau protein and total Tau protein levels in brain homogenates. C. Representative images and quantification of synaptic proteins (PSD95, Synapsin-1, SNAP25 and VAMP1). n=7-9 per group; * indicates P < 0.05; ** indicates P < 0.01. The scale bars in A are 500 µm. Data are represented as mean ± SEMs.
Figure 3

Terazosin treatment improved behaviour deficits in AD mice. A and B. Escape latency during platform trials and number of crossing platform and time spent in target quadrant in Morris water maze test. C. Distance travelled and the ratio of time spent in central and peripheral areas in the open-field test. D. Spontaneous alterations in the Y maze test. E. Representative tracking images in the open-field test. n=8 per group; * indicates P < 0.05; ** indicates P < 0.01. Data are represented as mean ± SEMs.
Terazosin treatment attenuated brain Aβ burden in AD mice. A. Immunostaining and quantification of Aβ plaques stained with 6E10 and Congo red in the neocortices and hippocampus of AD mice treated with saline or terazosin. B. Representative images of immunostaining of 1A4 (green) and 6E10 (red) as well as the number of cerebral amyloid angiopathy (CAA) number visualized by Congo red staining. C. Comparison of Aβ40 and Aβ42 levels in the TBS and SDS fractions of brain homogenates between the
saline and terazosin group. D and E. Representative western blots and quantitative analysis of the levels of APP-metabolizing proteins and Aβ-degrading enzymes as well as Aβ-transporting receptors brain homogenates. n=7-8 per group; * indicates P<0.05 and ** indicates P<0.01. Scale bar in A is 500 µm and in B is 25µm. Data are represented as mean ± SEMs.

Figure 5
Terazosin treatment attenuated AD-related pathogenesis. A. Representative immunostaining images of NeuN&Tau231, NeuN&MAP2 and NeuN&Caspase3. B. Representative western blots and quantitative analysis of phosphorylated Tau protein (PS199 and PS396) and total Tau protein levels in brain homogenates. C. Representative images and quantification of synaptic proteins (PSD95, Synapsin-1, SNAP25 and VAMP1). D. Quantification of neurons (NeuN) and dendrites (MAP2) in the CA1 region of the hippocampus and neural apoptosis (Caspase3) in the CA3 region of the hippocampus. E. Representative immunostaining images of CD68&NeuN and GFAP&NeuN. F. Immunostaining of astrocytes and microglia with GFAP and CD68 in the neocortices and hippocampus of mice treated with saline or terazosin. n=6-9 per group; * indicates P<0.05; ** indicates P<0.01. The scale bars in A and E are 50 µm. Data are represented as mean ± SEMs.

Figure 6

Terazosin reduced Aβ production through decreasing the expression of BACE1 in vitro. A. Representative western blots and quantitative analysis of the levels of APP-metabolizing proteins and Aβ levels in cell medium and lysate. B. Representative immunostaining images of BACE1 expression of SH-SY5Y-APP695 cells. C. Representative western blots and quantitative analysis of the levels of Aβ-producing enzymes and LRP1. D. Representative western blots and quantitative analysis of the levels of phosphorylated
GSK3β and GSK3β. n=6-9 per group; * indicates P<0.05; ** indicates P<0.01. The scale bars in A and E are 50 μm. Data are represented as mean ± SEMs.

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