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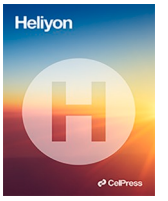

Research article

Differential involvement of central and peripheral catecholamines between Alzheimer's disease and vascular dementia

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ABSTRACT

Background and aim: The important role of catecholamines has been gradually emphasized in the pathogenesis of neurodegenerative process. As the most prevalent form of cognitive dysfunction, Alzheimer's disease (AD) and vascular dementia (VaD) have the distinct pathological features and pathogenic mechanisms, however, the differential involvement of central and peripheral catecholamines between AD and VaD was still unclear.

Methods: Triple-transgenic AD (3 × Tg-AD) mice and chronic cerebral hypoperfusion (CCH) in rats induced by two-vessel occlusion (2VO) were used as the AD and VaD model in this study, respectively. The concentrations of catecholamines (dopamine, epinephrine and norepinephrine) and their metabolites (3-methoxytyramine, metanephrine and normetanephrine) in serum and five brain regions (hippocampus, cortex, corpus striatum, thalamus and pons) from 3 × Tg-AD mice and 2VO rats were quantitatively determined by liquid chromatography-tandem mass spectrometry (LC-MS/MS) assay.

Results: High expression and distribution of hippocampal dopamine, and epinephrine and norepinephrine in the cortex and thalamus were found in the early 3 × Tg-AD model, whereas chronic cerebral hypoperfusion induced by 2VO mainly affected the central noradrenergic and noradrenergic system, but not dopaminergic system. The increased serum levels of catecholamines were investigated in the 2VO rats, but not in the 3 × Tg-AD mice.

Conclusion: The differential expression and distribution of central catecholamines and their metabolites suggests the distinct catecholamines-related pathogenesis between AD and VaD. Peripheral catecholamine surge may be involved in the development of VaD, and the treatment strategy to prevent or reverse the effects of peripheral catecholamines may be protective for VaD.

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1. Introduction

The catecholamines are comprised of dopamine, epinephrine, and norepinephrine, primarily associated with the central nervous system (CNS) as neurotransmitters or neuromodulators. Dysfuntion of catecholaminergic system is involved in the development of neurodegenerative diseases, which may be used as a potential therapeutic target for cognitive impairment [1]. Cognitive impairment appears as a core feature of dementia, including its most prevalent form, Alzheimer's disease (AD) and vascular dementia (VaD). Nevertheless, they have distinct pathological features and pathogenic mechanisms [2]. Along with the amyloid plaques and neurofibrillary tangles, neurodegeneration with brain atrophy hallmarks AD neuropathology [3]. While, VaD involves the impairment of memory and cognitive function as a consequence of cerebrovascular diseases [4]. Abnormal dopamine and norepinephrine expression has been reported in AD brain, however, the involvement of central catecholaminergic system in VaD was still mysterious [5,6]. Moreover, the catecholamines also play a vital role as circulating hormones released from hypothalamic-pituitary-adrenal (HPA) axis and sympathetic nerves. They may be acitivated after ischemic brain injury, called as catecholamine surge [7,8]. Whether the expression profile of peripheral catecholamines in cerebrovascular event-induced VaD are different from that in AD is also interesting.

To further understand the mechanism of catecholaminergic disruption in cognitive impairment, we explored the expression characteristics of central and peripheral catecholamines in AD and VaD in this study. Using a sensitive and specific liquid chromatography-tandem mass spectrometry (LC-MS/MS) assay, catecholamines and their metabolites in serum and brain tissues were quantitatively analyzed from the animal model of AD and VaD, respectively. The differential expression profiles of catecholamines and the underlying possible mechanism between AD and VaD were further assessed.

2. Research design and methods

2.1. Animals and ethics

All animal procedures and protocols were approved by the Research Animal Resources and Care Committee of Zhongnan Hospital of Wuhan University (No. ZN2021020) and performed in accordance with the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. Triple-transgenic Alzheimer's disease (3 × Tg-AD) mice harboring *PSEN1 M146V* knockin, *APPswe* and *TauP301L* transgenes were purchased from the Jackson Laboratory (MMRRC stock #34830, RRID: MMRRC_034830-JAX). Homozygous adult male 3 × Tg-AD mice (25–30 g, 6 months of age) were used and compared to age- and sex-matched wild-type (WT) mice (C57BL/6J), which are derived from the original mouse line with the same genetic background. Male mice were chosen to avoid additional factors affecting AD, such as hormones in female. Male Sprague-Dawley (SD) rats (220–260 g, 8–10 weeks of age) were obtained from the SPF Biotechnology Co., Ltd (Beijing, China) and used in our study. All animals were housed in a controlled environment (24 ± 2 °C, 12 h light and dark cycles) and received standard laboratory food and filtered clean water *ad libitum*.

2.2. Chronic cerebral hypoperfusion model

We used chronic cerebral hypoperfusion (CCH) in rats induced by two-vessel occlusion (2VO) operations as a VaD model, as described in our previous research [2]. Briefly, rats were anesthetized with 1 % pelltobabitalum natricum (40 mg/kg). After a ventral midline incision in the neck area, the left and right common carotid arteries (CCAs) were carefully isolated from the neighboring vagus nerve and ligated permanently with 4-0 silk suture to create the hypoperfusion and 2VO paradigm. In the sham-operated group, rats were subjected to similar surgical procedures without ligation. During the surgery, the body temperature of rats was kept at 37 ± 1 °C using a heating pad. After recovery from anesthesia, rats were returned to tha animal housing facility until further experiments.

From 15 min before to 15 min after the 2VO, cerebral blood flow (CBF) in the hippocampal CA1 region was measured using a laser doppler velocimetry, as described previously [9]. Briefly, a skull hole was drilled into the left side of hippocampal CA1 region (anteroposterior = 4.8 mm, mediolateral = ±2.5 mm, dorsoventral = −3.5 mm) and a 0.45 mm diameter laser doppler probe (moorVMS-LDF1, UK) was used to measure CBF from this hole into the hippocampus. When the CBF was stable, continuous recording was performed for 5 min using Perisoft software. Hippocampal blood flow was presented as the percentage of the postoperative value relative to the peroperative value.

2.3. Novel object recognition test

To evaluate the short-term memory, the novel object recognition (NOR) test was carried out in an opaque open-field box (50 cm × 40 cm × 40 cm), as described previously [10]. Briefly, two identical objects (A and B) were put at opposite ends of a single wall. On the first day, the rat was allowed to freely explore both items for 10 min. On the second day, the object B was replaced by a brand-new object C, and the rat was placed into the box again to freely investigate for 10 min. Nosing and smelling at an object within a distance less than 2 cm was defined as exploring it. The objects and the field were cleaned with 75 % ethanol between each experiment. A computerized video imaging analysis system (AVTAS Animal Video Tracking Analysis System, China) was used to record the trace of the rat's exploration and the total time spent on each object. The percentage of the recognition index (RI) was calculated using the following formulae [11]:

RI (%) = [time spent on the new object/(time spent on the new object + time spent on the familiar object)] × 100 %

2.4. Morris water maze test

The morris water maze (MWM) test is a classical task widely used to assess spatial learning and reference memory in rodents [12]. The MWM device consisted of a cylindrical black pool (150 cm in diameter and 60 cm in height) into which water was poured to a depth of 32 cm at room temperature and dyed using black ink. The maze was evenly divided into four quadrants, and a cylindrical platform (10 cm in diameter and 30 cm in height) was placed in the center of a certain quadrant. The probe train aims to assess the strength and persistence memory of the original platform location. From day 1 to day 5, each animal was placed into the water at four different locations on the opposite side of the platform. Animals were allowed to freely search the hidden platform in 60 s. After each trial, all animals were allowed to observe the surroundings on the platform for 15 s. The time spent in finding the platform was recorded as "escape latency". On day 6, the platform was removed and each animal received a 60 s probe test in the maze. The time spent swimming in the target quadrant and the number of times to cross the platform were recorded using a computerized video imaging analysis system (AVTAS Animal Video Tracking Analysis System, China).

2.5. Immunohistochemistry

The sections from 3 × Tg-AD and WT mice were processed for immunocytochemical detection of β-amyloid and hyperphosphorylated Tau to confirm AD-like pathology. Briefly, the 3 × Tg-AD and WT mice were deeply anesthetized and perfused sequentially with 5 % PBS and 4 % paraformaldehyde for 5 min. Brains were extracted and fixed in 4 % paraformaldehyde for 24 h, then embedded in paraffin wax and sectioned into 4 μm coronal slices. For antigen retrieval, sections were incubated in EDTA buffer (pH 8.0) and treated with H_2O_2 to quench autofluorescence for 5 min, followed by blocking with BSA for 30 min. Then, sections were incubated overnight at 4 °C with primary antibodies against β-amyloid (1:50, Santa Cruz Biotechnology, sc-28365) or phospho-Tau (Ser202, Thr205) monoclonal antibody (AT8) (1:100, Thermo Fisher Scientific, MN1020). After three washes with PBS, the sections were incubated with biotinylated secondary antibodies, followed by Avidin-Biotin Complex (ABC)-HRP conjugates. The immune reaction was visualized using Diaminobenzidine (DAB) as the substrate, and nuclei was counterstained with hematoxylin.

2.6. Reagents and chemicals used for LC-MS/MS method

The reagents of dopamine (DA), epinephrine (Epi), norepinephrine (NE), metanephrine (MN), normetanephrine (NMN), and 3-methoxytyramine (3-MT), and the corresponding internal standards (IS) including DA-d4, Epi-d3, NE-d6, MN-d3, NMN-d3 and 3-MT-d4, were purchased from Calibra Diagnostics (Hangzhou, China). Formic acid, methanol, perchloric acid, dansyl chloride, methylene chloride and isoflurane of HPLC grade were bought from Sigma-Aldrich (TX, USA). Catecholamine Quality Control (QC) 1, 2 and 3 were also obtained from Calibra Diagnostics. Certain amount of calibrators, IS and QC samples as received was nitrogen dried and then dissolved in methanol/water (50 %, v/v) to prepare stock solutions. All standard solutions were aliquoted and stored at −80 °C until further use. The concentrations of test solutions for the six analytes were listed in Supplementary Table S1.

2.7. Sample preparation and extract procedure

The sample preparation were performed in 6-month-old homozygous male 3 × Tg-AD mice and the experimental rats at 8 weeks after 2VO surgery. Blood specimens from the retro-orbital sinus of the experimental animals were collected into tubes. After standing for 30 min, the blood samples were centrifuged for 10 min at 3,800 rpm. The sera were separated and allocated to tubes, and preserved at −80 °C for further analysis. Under deep anesthesia with 2–3% isoflurane, the forebrains of the experimental animals were removed quickly. The cerebral cortex (Cor), hippocampus (Hippo), corpus striatum (Cs), thalamus (Th) and pons regions were dissected separately and homogenized in 0.1 M perchloric acid (100 μl for 20 mg tissues). The tissue homogenates were clarified by centrifugation (10,000 g for 10 min), and the supernatants were separated for the further extract.

Catecholamine extraction liquid (Calibra Diagnostics Co., Ltd., Zhejiang, China) was applied to pretreat the samples, using the derivatization method. Briefly, 200 μl of serum or tissue homogenate supernatant (or calibrator/QC sample) was mixed with 20 μl IS working solution to be fully vortexed, and then the derivant dansyl chloride was added. The mixture were centrifuged at 15,000 rpm for 10 min, then placed in oven at 50 °C for 1 h. After derivatization, 200 μl of the supernatant was transferred to a 96-well supported liquid extraction (SLE) cartidges for standing 10 min. Further, 1.5 ml of methylene chloride was added into SLE cartidges, then the eluent was collected and dried by nitrogen. Last, the samples were dissolved in 20 % methanol in water and centrifuged at 4,000 rpm for 10 min, then 100 μl of the supernatant was transferred into a 96-well microtiter plate for the following LC-MS/MS assay.

2.8. LC-MS/MS conditions and quantitative measurement

Serum and tissue levels of catecholamines and their metabolites were quantified using LC-MS/MS assay at the Department of Clinical Laboratory at Zhongnan Hospital of Wuhan University. The methodological establishment and validation of LC-MS/MS for catecholamines were performed in accordance with the C62-A standard for clinical mass spectrometry by CLSI [13]. The analyses were conducted on a Jasper™ High-Performance Liquid Chromatography (HPLC) system coupled to an AB SCIEX Triple Quad™ 4500MD mass spectrometer with an electron spray ionization source in the positive ion mode. The chromatographic separation was achieved with a Phenomenex Kinetex Biphenyl (50 × 3.0 mm, 2.6 μm) column. The mobile phases consisted of 0.1 % formic acid in water (mobile phase A) and methanol (mobile phase B). Gradient elution was as follows: 0–1.2 min 75 % A, 25 % B; 1.2–4.5 min linear

gradient to 10 % A, 90 % B; 4.5–5.0 min equilibrate with 75 % A, 25 % B. 30 μl of sample was injected at a flowrate of 0.6 ml/min. The multiple reaction monitoring (MRM) transitions used for DA was 853.2 > 619.2 as quantifier and 857.0 > 623.2 as qualifier, for Epi was 883.2 > 537.2 as quantifier and 886.2 > 537.2 as qualifier, for NE was 869.2 > 537.1 as quantifier and 875.2 > 542.2 as qualifier, for 3-MT was 634.2 > 400.2 as quantifier and 638.2 > 404.2 as qualifier, for MN was 664.2 > 567.1 as quantifier and 667.2 > 570.2 as qualifier, and for NMN was 650.2 > 553.1 as quantifier and 653.2 > 556.2 as qualifier. MultiQuant™ MD 3.0.2 software was used to quantify the concentrations of the six analytes. The area ratios of the analyte to the internal standard peak of the six calibration standards were plotted to generate the calibration curve for each set of samples. The plot used linear regression with $1/x^2$ weighting, and the correlation coefficient of determination (R^2) greater than 0.99 for all the analytes (Supplementary Table S2). Three levels of QC samples were included in each experiment.

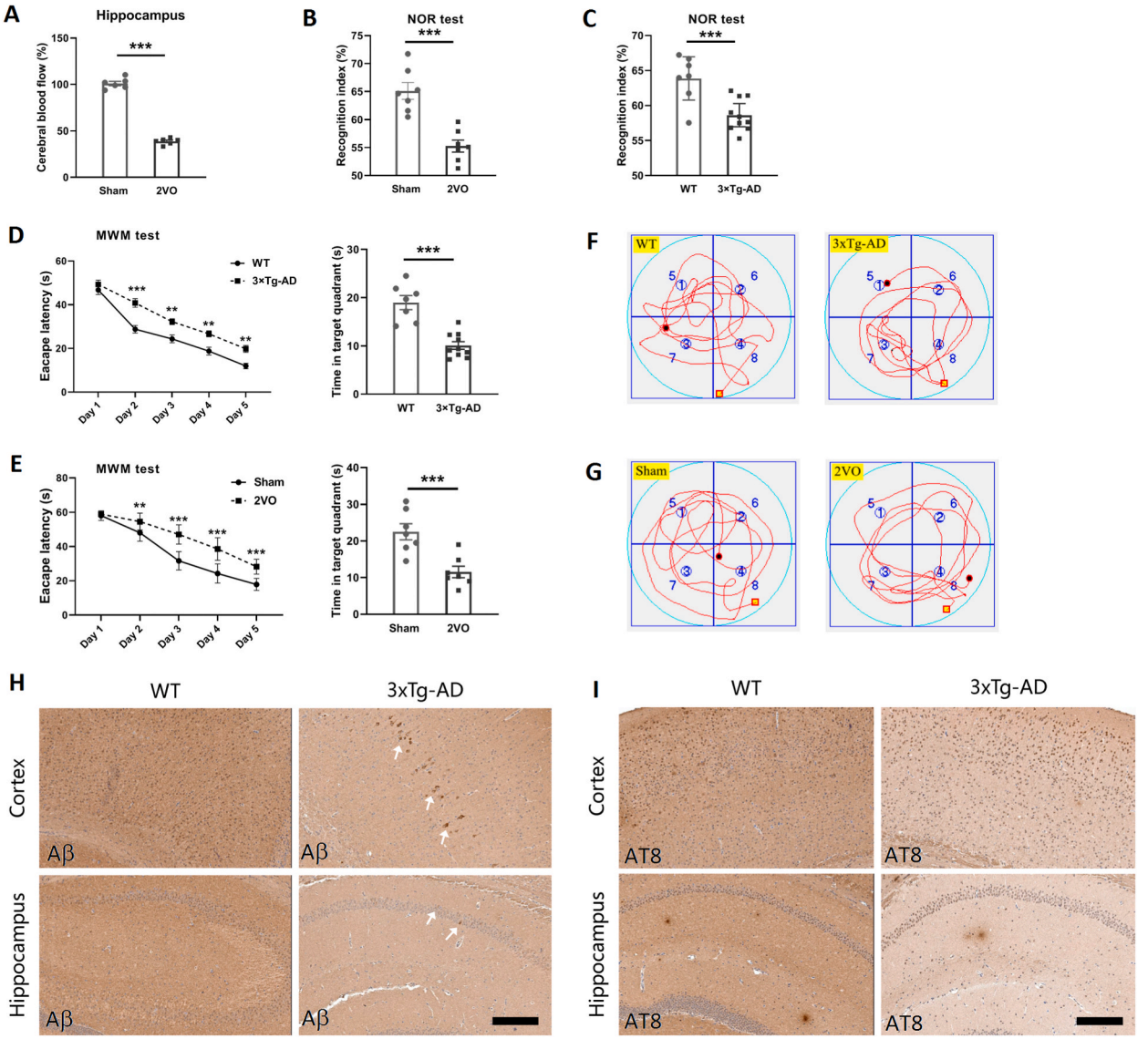

Fig. 1. Cognitive impairment in the animal model of AD and VaD. (A) The hippocampal blood flow in sham ($n = 7$) and 2VO ($n = 7$) groups was recorded using a laser doppler velocimetry, and presented as the percentage of the postoperative value relative to the peroperative value. (B) The recognition index of NOR test in sham ($n = 7$) and 2VO ($n = 7$) groups. (C) The recognition index of NOR test in WT ($n = 7$) and 3 × Tg-AD ($n = 10$) groups. (D) The escape latency and time in target quadrant of MWM test in WT ($n = 7$) and 3 × Tg-AD ($n = 10$) groups. (E) The escape latency and time in target quadrant of MWM test in sham ($n = 7$) and 2VO ($n = 7$) groups, respectively. (F, G) The representative swimming track of AD and VaD model animals, compared with the corresponding control groups. (H, I) Immunoreactivity of Aβ and AT8 in the cortex and hippocampus regions of 6-month-old WT and 3 × Tg-AD mice. scale bar, 200 μm. The data (A–E) were presented as means ± SEM and analyzed by Student's *t*-test. $^{**}p < 0.01$, $^{***}p < 0.001$.

2.9. Statistical analysis

GraphPad Prism 8.0 software (GraphPad Software Inc., La Jolla, CA, USA) were used for statistical analysis. All data were represented as mean ± SEM. The significance of the generated data was determined using Student's *t*-test was applied to analyze differences between two groups, and one-way analysis of variance (ANOVA) followed by Tukey's *post hoc* test was used for multiple comparisons. Statistical differences were considered significant when $p < 0.05$ and very significant when $p < 0.01$.

3. Results

3.1. Cognitive impairment in the early AD and VaD

The recapitulation of salient features of AD in 3 × Tg-AD mouse model clarifies the relationship between Aβ, synaptic dysfunction and tangles, providing a valuable model for AD research [14]. Here, the 6-month-old male 3 × Tg-AD mice were used, which genotype has been identified to be homozygous. The CCH surgery induced by 2VO in rats is considered as a suitable animal model to mimic chronic brain hypoperfusion status, which is proved to be a risk factor to precede VaD [15]. In this study, the hippocampal blood flow in 2VO rat model was reduced by nearly 65 %, indicating that the CCH model induction was successful (Fig. 1A). Further, the NOR test and MWM test were performed to evaluate the cognitive impairment in animal model of the early VaD or AD. For the NOR test, the RI value in 2VO rats was significantly lower than that in the sham group (Fig. 1B). The RI value in 3 × Tg-AD mice also showed the similar difference, compared with WT mice (Fig. 1C). For the MWM test, there was an obvious difference in escape latency time during the training period and time spent in target quadrant during the probe trial period between 3 × Tg-AD or 2VO and the respective corresponding control group (Fig. 1D–E). The representative swimming trace of experimental animals was shown in Fig. 1F & G. Compared to the WT group, homozygous 3 × Tg-AD mice showed Aβ immunoreactivity in both the cortex and hippocampus regions

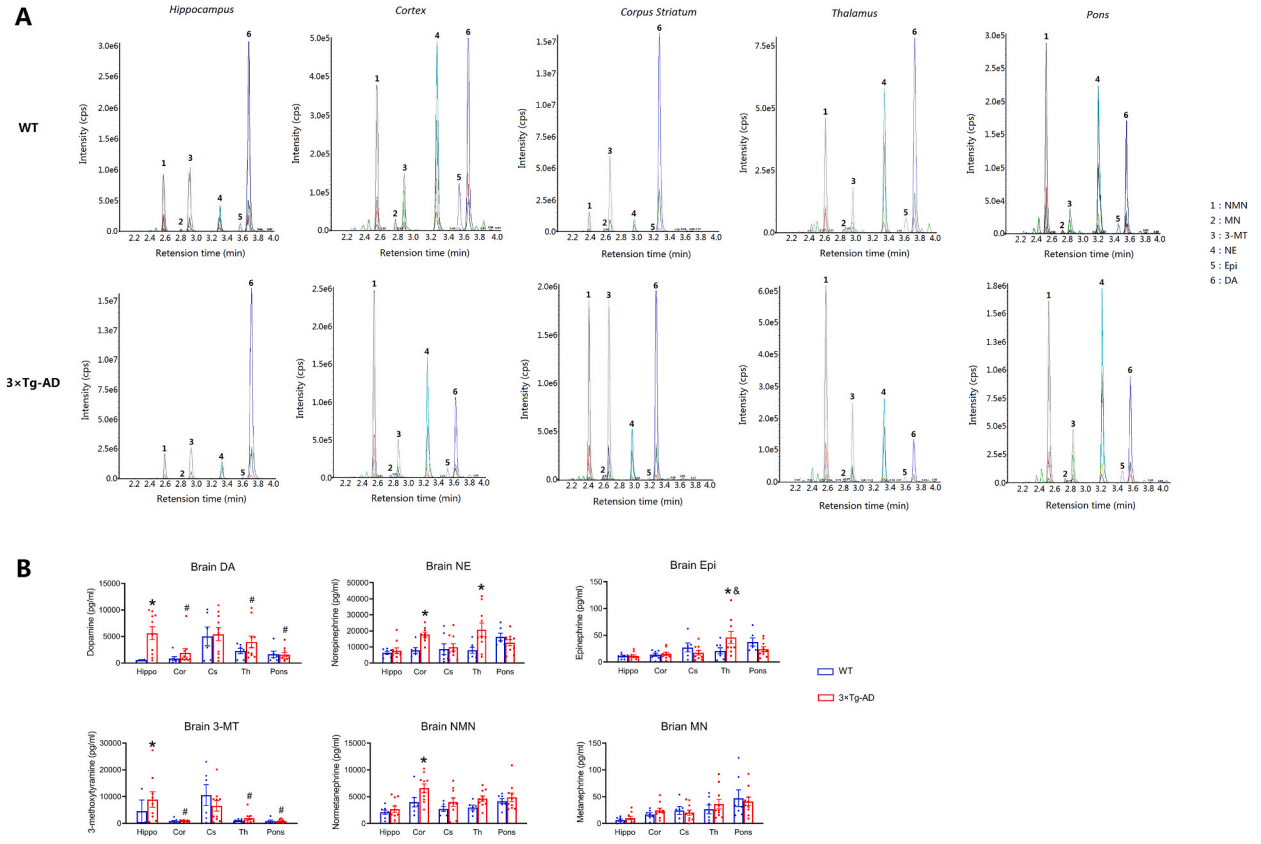

Fig. 2. The expression profiles of catecholamines and their metabolites in the differential brain regions in 3 × Tg-AD mice. (A) The representative chromatogram showing signals of catecholamines (DA, NE and Epi) and their metabolites (3-MT, NMN and MN) in the different regions of mouse brain, including hippocampus (Hippo), cortex (Cor), corpus striatum (Cs), thalamus (Th) and pons. *x* axis, retension time (s); *y* axis, intensity (cps). (B) The statistics of catecholamines concentrations from WT (n = 7) and 3 × Tg-AD mice (n = 10). Quantification was performed on the basis of peak area ratios of the targeted analyte compared to its corresponding internal standard. Data are shown as means ± SEM, $^{*}p < 0.05$ compared with the corresponding WT groups; $^{\#}p < 0.05$ compared with the hippocampus region in the 3 × Tg-AD mice; $^{\&}p < 0.05$ compared with the other four regions in the 3 × Tg-AD groups.

(Fig. 1H). In contrast, AT8 immunoreactivity, which detects phosphorylated Tau at residues S202/T205, was absent in these regions (Fig. 1I). These observations indicated that Aβ deposition preceded Tau pathology in 3 × Tg-AD mice, which was consistent with the previous study [16]. Collectively, these data suggest that the cognitive impairment occurs in the animal model of early AD and VaD.

3.2. The expression profiles of catecholamines and their metabolites in the different brain regions in AD mice

DA can be enzymatically metabolized via catechol-O-methyl transferase (COMT), which replaces a hydroxyl group with a methoxy group to form 3-MT. 3-MT has been found to be involved in the progressive neurodegeneration. MN and NMN are the two catechol 3-O-methylated metabolites of Epi and NE, respectively [17]. Considering the fast metabolic characteristics of catecholamines, the function analysis of catecholaminergic systems in cognitive impairment-related disorders was combined with their metabolites in our study.

The origin of NE in the CNS is mainly from the locus coeruleus, which resides in the brainstem's dorsal pons and is linked to the hippocampus, thus being important for memory formation [18,19]. Epi can be released in response to neuronal activation, which is synthesized from NE via the methylation of phenylethanolamine N-methyltransferase (PNMT) [20]. DA is an amine formed by the precursor chemical L-DOPA mainly in cortical circuits and basal ganglia, which can interact with its receptors expressed especially in the hippocampal dentate gyrus in the CNS [21]. The dysfunction of the fronto-striato-thalamic system was recently suggested to be associated with the pathogenesis of poor cognitive performance [22]. Taken together, the five critical brain regions were chosed to analyze the expression and distribution of catecholamines and their metabolites in this study, including the cerebral cortex, hippocampus, corpus striatum, thalamus and pons regions.

The normal expression characteristics of catecholaminergic system in the above five brain regions were first analyzed in the WT mice by LC-MS/MS. DA and its metabolite 3-MT showed the relatively high expression and distribution in the corpus striatum region. NE was mainly distributed in the pons region, the level of which was almost 2.5 fold higher than that in the hippocampus. The distribution of Epi and its metabolite MN was similar with NE, but their concentrations were rather low, just the approximately

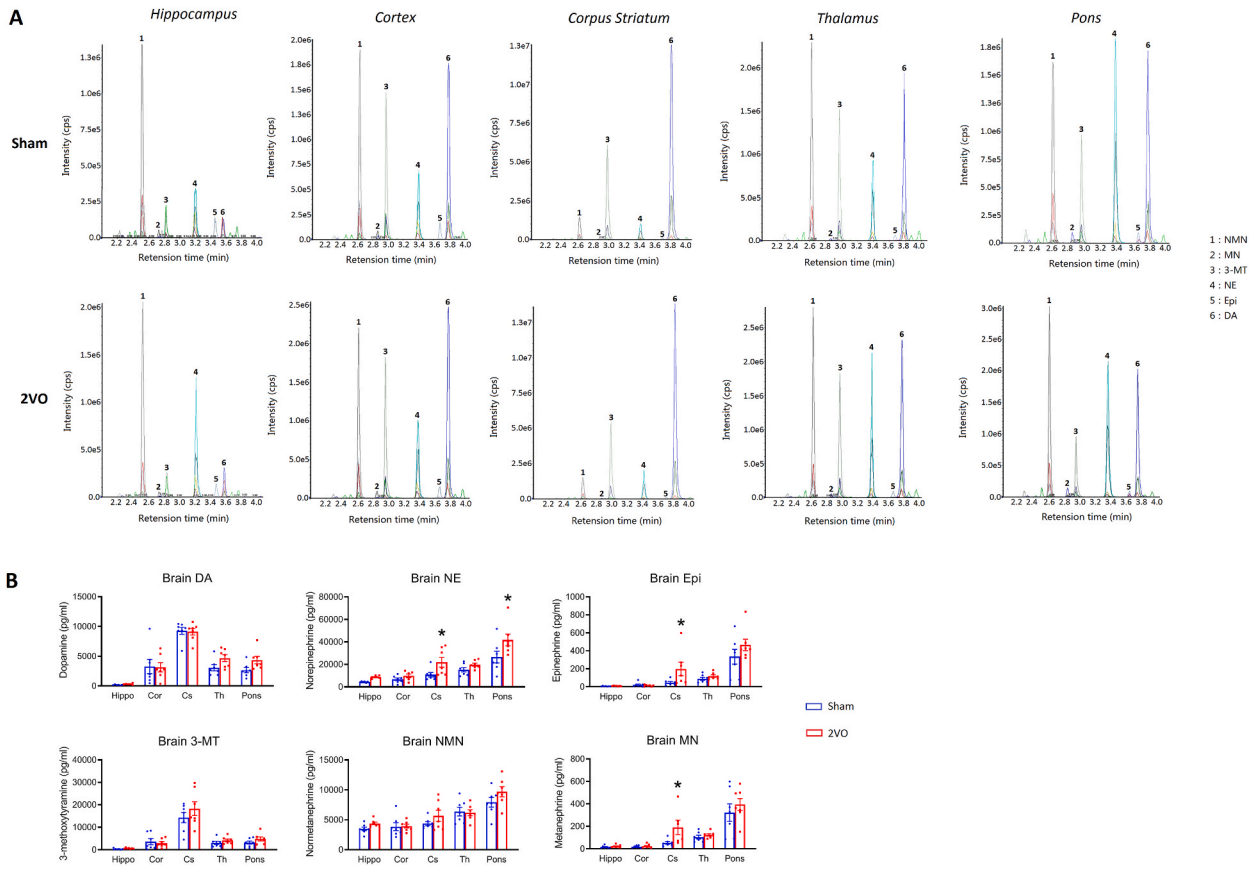

Fig. 3. The expression profiles of catecholamines and their metabolites in the differential brain regions in 2VO rats. (A) The representative chromatogram showing signals of catecholamines (DA, NE and Epi) and their metabolites (3-MT, NMN and MN) in the different brain regions of rat brain, including hippocampus (Hippo), cortex (Cor), corpus striatum (Cs), thalamus (Th) and pons. *x* axis, retension time (s); *y* axis, intensity (cps). (B) The statistics of catecholamines concentrations from sham (n = 7) and 2VO model rats (n = 7). Quantification was done on the basis of peak area ratios of the targeted analyte compared to its corresponding internal standard. Data are shown as means ± SEM, $*p < 0.05$ compared with the corresponding sham groups.

thousandth levels of NE (Supplementary Fig. S1). Compared with the WT mice, the changes in the levels and distribution of catecholamines and their metabolites occurred in the 3 × Tg-AD mice. There was a significant elevation of DA and 3-MT concentrations in the hippocampus in the 3 × Tg-AD mice. The levels of NE in the cortex and thalamus were increased, but the elevation of its metabolite NMN was only detected in the cortex region. The level of Epi was obviously elevated in the thalamus than that in the other four regions in the 3 × Tg-AD mice, however, its metabolite MN was still highly distributed in the pons region (Fig. 2). The detailed quantitative data were listed in Supplementary Table S3. Thereby, the changes of hippocampal dopamine system, norepinephrine and epinephrine system in the cortex and thalamus may be closely related to the AD pathogenesis.

3.3. The expression profiles of catecholamines and their metabolites in the different brain regions in VaD rats

To explore the differential expression profiles for central catecholamines between AD and VaD, we further performed the quantitative analysis for catecholamines and their metabolites in the rat 2VO model and the corresponding sham group. In the sham groups, the highly expression of DA in the corpus striatum and NE/Epi in the pons region was detected, accompanied with their metabolites. The distribution was basically consistent with that in the WT mice (Supplementary Fig. S2). In the 2VO group, no obvious changes of DA and 3-MT levels were found in the five brain regions. Except for the continuous elevation of NE in the pons, the concentrations of NE/Epi and MN in the corpus striatum were also increased, compared with the sham group (Fig. 3). The detailed quantitative data were listed in Supplementary Table S4. Thus, the process of CCH-induced VaD may mainly affect the noradrenergic and adrenergic systems in the CNS, but not dopaminergic system.

3.4. The serum levels of catecholamines and their metabolites in the animal model of AD and VaD

As the important circulating hormones, the expression characteristics of catecholamines and their metabolites in serum were further analyzed by LC-MS/MS under the condition of AD and VaD. Compared with the WT mice, the serum levels of catecholamines and their metabolites did not show the significant increase or decrease in the 3 × Tg-AD mice (Fig. 4). In contrast, the serum levels of DA, Epi and NE in the 2VO groups were higher than those in the corresponding sham groups, despite without the accompanying changes of their metabolites (Fig. 5). Hence, the effect of circulating catecholamines may be involved in the development of VaD.

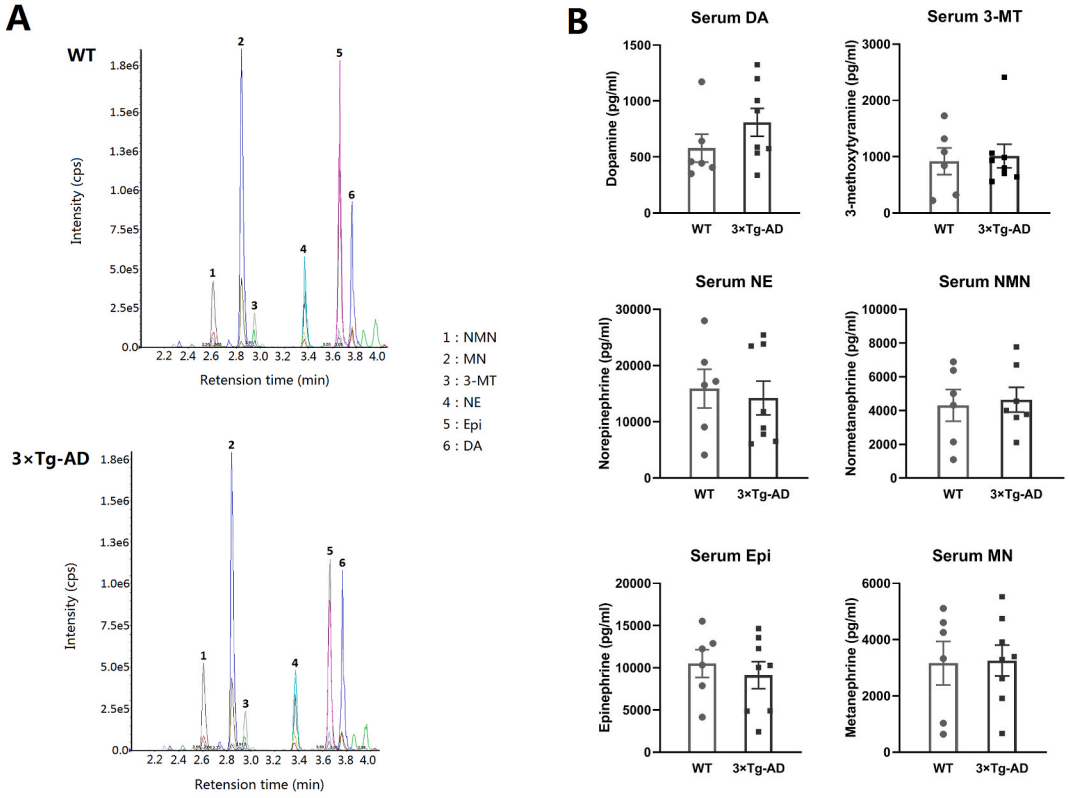

Fig. 4. The serum expression profiles of catecholamines and their metabolites in 3 × Tg-AD mice. (A) The representative chromatogram showing signals of serum catecholamines (DA, NE and Epi) and their metabolites (3-MT, NMN and MN) in WT and 3 × Tg-AD mice. (B) The statistics of serum catecholamines concentrations from WT (n = 7) and 3 × Tg-AD mice (n = 10). Quantification was done on the basis of peak area ratios of the targeted analyte compared to its corresponding internal standard. Data are shown as means ± SEM and compared by Student's *t*-test.

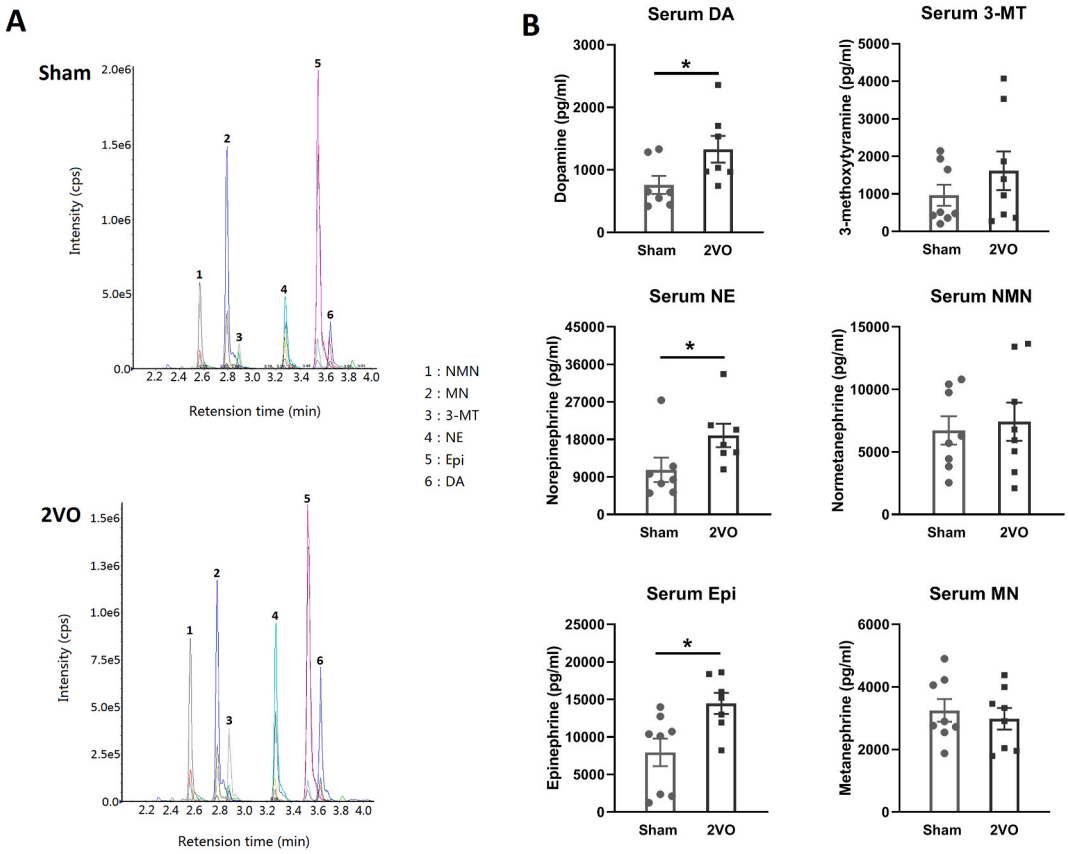

Fig. 5. The serum expression profiles of catecholamines and their metabolites in 2VO rats. (A) The representative chromatogram showing signals of serum catecholamines (DA, NE and Epi) and their metabolites (3-MT, NMN and MN) in the Sham and 2VO rats. (B) The statistics of serum catecholamines concentrations from sham (n = 7) and 2VO model rats (n = 7). Quantification was done on the basis of peak area ratios of the targeted analyte compared to its corresponding internal standard. Data are shown as means ± SEM, $^{*}p < 0.05$ compared with the corresponding sham groups.

4. Discussion

Due to the distinct pathological features and pathogenic mechanisms, the difference between AD and VaD about the expression and distribution of catecholamines was still unclear. In this study, catecholamines and their metabolites were quantitatively analyzed using the LC-MS/MS assay. First, the differential expression profiles of catecholamines and their metabolites were shown in the brain from animal model of AD and VaD. For the central dopamine system, i) dopaminergic neurons originate from the substantia nigra and the ventral tegmental area, and the dopaminergic neuronal loss was confined to the ventral tegmental area in the AD model [23,24]. However, we found that the concentrations of DA and its metabolite 3-MT in the corpus striatum were obviously high under the healthy condition, but in the 3 × Tg-AD mouse model with Aβ pathology, the levels of hippocampal DA and 3-MT were significantly elevated, which may be due to the release or projection that originates in other brain regions, such as nigrostriatum. The reason for the elevated dopamine system in AD needs to be further elucidation. ii) The DA receptors are expressed especially in the hippocampal dentate gyrus (DG) and subventricular zone (SVZ), but the increased tonic release of DA may resulting in the down-regulation of DA receptors [25,26]. Here, we found a significant elevation of DA and 3-MT in the hippocampus in the 3 × Tg-AD mice, which may lead to the down-regulation of hippocampal DA receptors and further increase hyperexcitability. iii) No significant elevation of DA and 3-MT was found in the five detected brain regions in the 2VO model compared to the sham control. Maybe, the process of AD could affect the expression and distribution of dopaminergic neurotransmission in the CNS, linked with Aβ pathology, while the process of CCH-induced VaD did not.

Noradrenergic neurons are mainly clustered in small nuclei located in the pons and lateral reticular formation of the medulla. Among, the most of NE are produced in the locus coeruleus (LC) [27]. The reduction in the number of noradrenergic neurons from the LC has been observed in the initial postmortem analyses of brains from AD patients [28]. According to our results, the pons region was responsible for the most of NE produced in the brain from the normal WT mice or sham-treated rats. In the 3 × Tg-AD mice, the abnormal levels of NE were detected in the cortex and thalamus regions, while the level in the pons did not present the obvious decrease. In the 2VO rats, the high levels of NE were distributed in the corpus striatum. The differential distribution and expression

between the AD and VaD model revealed that NE may activate adrenergic receptors to generate the distinct effects, nevertheless, controversial studies about NE have pointed out both to neurotoxic and neuroprotective activity in cognitive impairment [27]. Based on, the abnormality of central norepinephrine system in the early AD and VaD associated with the "fight or flight" response needs to be further verified. PNMT methylates NE to form Epi, which is present in a very high concentration in the adrenal, while a low concentration in brain stem [29]. However, our results also showed the similar abnormality of central epinephrine system in the early AD and VaD. Compared with those in the peripheral circulation, the levels of Epi in the five brain regions were rather low, but its

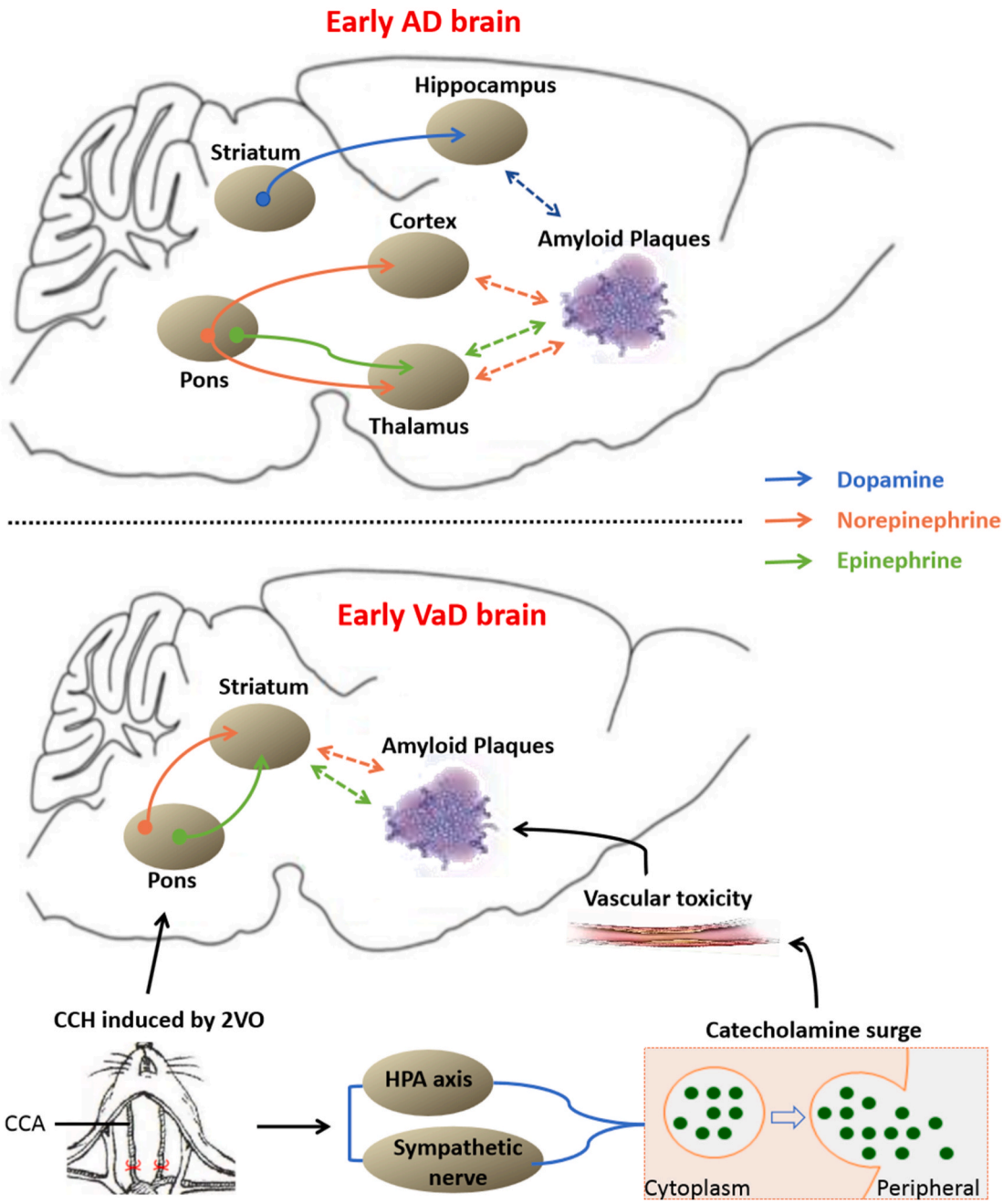

Fig. 6. A scheme of the differential involvement of central and peripheral catecholamines in the early AD and VaD animal model. High expression and distribution of dopamine system in hippocampus, and norepinephrine and epinephrine system in the cortex and thalamus may be closely related to Aβ pathology in the early AD model, whereas chronic cerebral hypoperfusion induced by 2VO may mainly affect the noradrenergic and epinephrine systems in the CNS, but not dopaminergic system, showing the distinct pathogenesis between AD and VaD. In the peripheral circulation, chronic cerebral hypoperfusion may induce catecholamine surge via the activation of hypothalamic-pituitary-adrenal (HPA) axis and sympathetic nerve, which may promote vascular toxicity and then aggravate the development of VaD pathology.

significance should not be ignored.

The differential expression profiles of catecholamines and their metabolites in the brain revealed the distinct involvement of catecholaminergic system in these two types of dementia, which may associated with the respective pathological features and pathogenic mechanisms. Meanwhile, we further investigated the other face of catecholamines as circulating hormones in the early AD and VaD. To be surprised, we found that the serum levels of DA, NE and Epi were all significantly increased in the 2VO model, while no obvious changes in the 3 × Tg-AD mice. Thus, we speculated that chronic cerebral hypoperfusion may promote the activation of HPA axis and sympathetic nerve, and the vascular toxicity induced by the increased catecholamines may lead to the further vascular occlusion and aggravation of cognitive impairment. The underlying mechanism for catecholamine surge induced by chronic cerebral hypoperfusion deserves further research. The involvement of peripheral catecholaminergic system in the AD process may be negligible. The symptoms of AD and VaD can be similar and often occur simultaneously as "mixed" dementia [30]. Detection of peripheral catecholamines may be helpful for the identification of VaD from AD or mixed dementia. Additionally, how to prevent or reverse the effects of peripheral catecholamines has been suggested to be the potential therapeutic target in brain injury and cerebral-cardiac syndrome [31,32]. For example, Phenoxybenzamine (PBZ) as a FDA approved treatment for humans and animals to reduce hypertension associated with adrenal tumors (pheochromocytoma), has been found to be a potential neuroprotective agent in brain injury [31,33]. In the future research, the effect of PBZ on VaD may be worth exploring.

There were still several limitations in our study. We just detected the concentrations of catecholamines and their metabolites for one time point in the early 3 × Tg-AD mice (6 months) and 2VO rats (8 weeks). Maybe a longitudinal investigation with the age and disease course would provide more information about the involvement of catecholamines and their metabolites in cognitive dysfunction. We found that the changes of the metabolites were not consistent with their corresponing catecholamines, which may be related to the differential distribution of COMT in the brain regions. Additional larger sizes are needed to validate our findings. As a proof-of-concept study, we can not address all questions arising from this initial work. However, our study aimed to suggest the differential involvement of catecholamines and their metabolites in the early AD and VaD, which may be the breach to further identify the unique mechanisms for cognitive impairment.

5. Conclusion

Taken together, high expression and distribution of hippocampal dopamine system, and norepinephrine and epinephrine system in the cortex and thalamus may be closely related to Aβ pathology in the early AD, whereas chronic cerebral hypoperfusion in the process of VaD may mainly affect the noradrenergic and epinephrine system systems in the CNS, but not dopaminergic system, showing the distinct pathogenesis between AD and VaD. In the peripheral circulation, catecholamine surge may be involved in the development of VaD (Fig. 6). Detection of peripheral catecholamines may be helpful for the identification of VaD from AD or mixed dementia, and the therapeutic strategy to prevent or reverse the effects of peripheral catecholamines may be protective for VaD, which should be concerned.

Consent statement

All co-authors have seen the submitted manuscript, agree with its content and approve of its publication, and that the material is not under consideration elsewhere. The authors have obtained informed consent from persons whose details are described in the manuscript that this information may be published, when applicable.

Ethics statement

The animal study was approved by Animal Ethics Committee of the Medical School of Wuhan University (No. ZN2021020). The study was conducted in accordance with the local legislation and institutional requirements.

Data availability statement

The data associated with our study has not been deposited into a publicly available repository, and our data will be made available on request.

CRediT authorship contribution statement

Xiao-Yue Hong: Visualization, Validation, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Siwei Li:** Methodology, Investigation, Formal analysis, Data curation. **Tian Li:** Methodology, Investigation, Formal analysis, Data curation. **Wei Chen:** Methodology, Investigation. **Yirong Li:** Supervision, Resources. **Zhuo Wang:** Writing – original draft, Supervision, Methodology, Conceptualization. **Yi Luo:** Writing – review & editing, Project administration, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

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