

## Original Article

# Effect of eplerenone on cognitive impairment in spontaneously hypertensive rats

Zhongqiao Lin<sup>1</sup>, Yan Lu<sup>1</sup>, Sheng Li<sup>1</sup>, Yiyang Li<sup>2</sup>, Han Li<sup>1</sup>, Lin Li<sup>1</sup>, Lei Wang<sup>1</sup>

<sup>1</sup>Department of Geriatrics, Third Hospital of Shanxi Medical University, Shanxi Bethune Hospital, Shanxi Academy of Medical Sciences, Taiyuan 030032, Shanxi, China; <sup>2</sup>Department of Physiology, Key Laboratory of Cellular Physiology, Ministry of Education, Shanxi Medical University, Taiyuan 030001, Shanxi, China

Received January 20, 2022; Accepted May 19, 2022; Epub June 15, 2022; Published June 30, 2022

**Abstract:** Objective: The present study aimed to determine the effect of blocking brain mineralocorticoid receptor on cognitive impairment in spontaneously hypertensive rats and its intracellular changes. Methods: 12-week-old male spontaneous hypertensive rats (SHR) and Wistar Kyoto (WKY) rats were given eplerenone (EPL, 30 mg/Kg/d or 100 mg/Kg/d) or pure water via oral gavage daily for 8 weeks. Effects of blocking brain mineralocorticoid receptor (MR) on cognitive function were examined through cognitive behavioral experiments. The morphology of hippocampal neurons was observed. Synaptic proteins and autophagy levels were detected by western blot. Results: The results showed decreases in both short-term working memory and long-term spatial learning and memory ability, hippocampal neuron damage, and reduced expression of synaptic proteins in the SHR-Veh group. Impaired autophagy was found in the SHR-Veh group as evidenced by decreased expression levels of Beclin-1 protein and a defect in P62 degradation. These abnormalities were reversed by eplerenone, either the high dosage or low dosage. Reduced cognitive dysfunction and enhanced autophagy in hippocampal neurons in both SHR-EPL30 group and SHR-EPL100 group were independent of lowering blood pressure. Conclusion: Eplerenone improves cognitive deficits observed in SHRs, and increases autophagy in hippocampal neurons of SHRs, which suggests a new site of MR antagonists in treatment of hypertension-related cognitive impairment.

**Keywords:** Hypertension, cognitive impairment, mineralocorticoid receptor, autophagy, spontaneous hypertensive rat

## Introduction

Hypertension is a cardiovascular disease characterized by elevated systemic arterial blood pressure that can harm multiple organs [1]. It is a major burden for the global population, with an estimated 1.56 billion people suffering from hypertension by 2025 [2]. An important feature of hypertensive brain damage is cognitive impairment, particularly a reduction in learning and memory ability, which has been observed both in patients and in several hypertensive rat models [3-5]. According to several cross-sectional studies, cognitive impairment happens in more than 1/4 of hypertensive patients, posing a significant burden on the individual, family, and society [6, 7]. Learning and memory abilities are the key functions of the hippocampus. The damage to hippocampal neurons in CA1 and dentate gyrus (DG) regions has been seen

in spontaneous hypertensive rats (SHRs) [8]. However, current studies have mainly concentrated on alleviating pathogenic abnormalities in cerebral arteries, with few investigations on intracellular changes in neurons and neuron protection. As a result, more emphasis must be given to hippocampal neurons in order to provide suitable treatment and improve the quality of life of hypertensive patients with cognitive dysfunction.

Mineralocorticoid receptor (MR) is a nuclear receptor that binds aldosterone and was previously thought to be important only in regulating water and salt balance. Recent studies have demonstrated that mineralocorticoid receptors are expressed in both human and animal brains, especially in the hippocampus [9, 10]. Clinical investigation showed that increased aldosterone levels were linked to a higher risk

## Effect of eplerenone on cognitive impairment

of impaired cognitive function in hypertensive patients [11, 12]. Some animal research found that mice with deletion of brain MRs showed a defect of neurogenesis in hippocampal granular cells as well as impaired learning and memory functions [13, 14]. Blockade of brain MRs using spiro lactone in healthy humans also caused a decline in spatial memory [15]. In both animal research and clinical trials, MR was essential for normal cognitive function, while spontaneously hypertensive rats with abundant MR expression in the hippocampus had cognitive decline [3, 16]. This opposing phenomenon may indicate that in the pathologic process of hypertension, MR activation leads to neuronal damage, resulting in a decline in cognitive function. Whether blockade of MR alleviates hippocampal neuron damage in hypertension is worth exploring.

Autophagy plays an important role in maintaining intracellular homeostasis as well as in degrading damaged organelles and abnormal proteins, and also participates in the normal activity of neurons [17]. Improvement of autophagy dysfunction could help protect the blood-brain barrier and ameliorate cognitive impairment in chronic cerebral hypoperfusion (CCH) rats [18]. Restoring autophagic flux in the brains of Alzheimer's disease (AD) mice improved spatial memory and synaptic plasticity [19]. Although autophagy deficits have been associated with cognitive impairment in several animal models, autophagy in the SHR brain and its contribution to learning and memory decline remain unknown. MR activation can either induce or impair autophagy. Blockade of MR by eplerenone down-regulated autophagy and collagen-1 expression in cultured mouse aortic smooth muscle cells [20]. Spironolactone restored autophagy activity and reduced adhesive capacity damage in podocytes [21]. However, little is known about whether MR induces or impairs autophagy in the brain. Thus, we hold the view that blockade of MR in the SHR hippocampus may have the effect of enhancing autophagy and protecting neurons.

Eplerenone is one of the second generation mineralocorticoid receptor antagonists with higher selectivity to MR and fewer side effects than spironolactone. In the present study, SHR and Wistar-Kyoto (WKY) rats were chosen for an animal model. The effects of eplerenone on

cognitive function and neuronal autophagy levels were observed and a possible molecular mechanism was investigated.

### Materials and methods

#### *Animals and drugs*

Male SHR and WKY rats were used in the present study. Both SHR and WKY rats were bought from Vital-River (Vital-River, China). Rats were housed 2-3 per cage with 12 hours light and 12 hours dark, with free access to water and food. 12-week-old SHRs were randomly divided into 3 groups: SHR-Veh (n = 12), SHR-EPL30 (Eplerenone 30 mg/Kg/d, n = 12), and SHR-EPL100 (Eplerenone 100 mg/Kg/d, n = 12). Another group of 12-week-old male WKY rats with normal blood pressure were set as the background group (n = 12). All the operations on rats were approved by the Animal Research Committee of Shanxi Bethune Hospital (No. 2021GLL128).

In SHR-EPL30 and SHR-EPL100 groups, eplerenone (EPL, Meilunbio, China) was given 30 mg/Kg/d or 100 mg/Kg/d, dissolved in 2 ml sterilized water, and ultrasound was used to make turbid liquid. The dosages of eplerenone and the dissolution method were based on previous studies [8, 22]. In SHR-Veh and WKY groups, sterilized water was given. Both eplerenone and equivalent sterilized water were given by oral gavage daily for 8 weeks [8].

#### *Blood pressure measurement*

Rat systolic blood pressure was measured non-invasively by the tail-cuff method before the experiments and at 1 week, 3 weeks, 6 weeks, and 8 weeks during oral gavage. Each time, rats received three consecutive measurements and the average value was taken.

#### *Serum potassium measurement*

Rat blood was collected by tail cutting method before the experiments and at 1 week, 4 weeks during oral gavage, and by abnormal aorta puncture when processing the tissue. The blood was placed stably at 4°C overnight and was centrifuged at 3000 rpm for 10 min at 4°C to separate the serum. After centrifugation, the serum was immediately stored at -80°C for future use.

## Effect of eplerenone on cognitive impairment

Potassium assay kit (C001-2-1, Jiancheng Bio, China) was used to measure serum potassium concentration. All operations were carried out in strict accordance with the instructions, and parallel wells were set to ensure an accurate result.

### *Y maze alternation test*

The Y maze alternation test was used to evaluate short-term working memory of rats [23]. The percentage of correct spontaneous alterations was the main evaluation criterion. The Y-maze apparatus consists of three arms (50 cm in length, 10 cm in width, and 30 cm in height) and a connecting area. The included angle was 120° to each arm. The inner wall of the Y maze was black and a camera was set about 1.5 m above the center to monitor the movement of the rats. The environment was quiet and rats were pre-adapted to the environment. Each rat was placed on the connecting area and had free access to three arms for 8 min. The apparatus was wiped with 75% ethanol solution and wiped dry before each trial. The number of arm entries and sequence of arm entries were recorded. Starting from the third arm entry, once a rat entered an arm different from the previous two, it was regarded as a correct arm entering. The method to calculate the percentage of correct spontaneous alterations was [(number of correct alternations)/(total number of arm entries -2)] \*100%.

### *Morris water maze test*

Morris water maze (MWM) test was used to evaluate the long-term spatial learning and memory ability of rats [24]. The apparatus was a black circular tank (150 cm in diameter and 50 cm in height), containing tap water (23 ± 1°C) and divided into four equal virtual quadrants: I, I, III and IV. Non-toxic black paint was added to water to make it opaque. Several geometric cues were placed on the curtain around the tank and a camera was set about 1.5 m above the center of water surface to monitor the movement of the rats. The environment was quiet and the light was suitable during the whole test. Morris water maze test included 3 parts: spatial navigation training, probe test, and visible platform test.

Spatial navigation training was continuously done for 5 days. The circle escape platform (10 cm in diameter) was placed on the center of the

III quadrant and 1 cm below the water surface. The rat was placed into the water facing the middle of the wall at one of the four quadrants and allowed to swim freely until it found and climbed onto the platform. Once the rat climbed onto the platform, it was allowed to stay for 10 s. If the rat failed to locate the platform within 120 s, it was guided to the platform and allowed to stay for 10 s. The escape latency was recorded as 120 s. Each rat performed four trials per day, with a different starting position for each trial.

The probe test was conducted on the sixth day. The circle escape platform was removed and the rat was placed into the opposite quadrant of the III quadrant. Each rat was allowed to swim freely for 120 s. The time spent in the III quadrant and the number of platform crossings were measured.

A visible platform test was performed to detect the visual ability of rats on the seventh day. The speed of swimming was measured to exclude rats with motor deficits. The tracks were monitored and analyzed by a computer-based video tracking system (Ethovision XT 15.0, Noldus, Netherlands).

### *Hematoxylin and eosin staining*

The rats in each group were randomly divided into two cohorts, one for hematoxylin and eosin (H&E) staining (n = 5) and another for western blot (n = 7). The 25% urethane was used to anesthetize rat in accordance with 0.6 ml per 100 g weight. After rats were deeply anesthetized, blood was collected by the abdominal aorta. Then the rat was perfused with cold 0.9% saline through the abdominal aorta and subsequently with cold 4% paraformaldehyde (PFA). Afterward, the brain was removed and fixed in PFA for 24 h. After fixation, the brain was sliced between the root of optic chiasma and the corpora quadrigemina to make a hippocampal sample. The samples were dehydrated with graded ethanols and embedded in paraffin. Coronal hippocampus sections (3.5 μm thick) were prepared and stained with H&E. Images were collected with an optical microscope (BX51, Olympus, Japan).

### *Western blot (WB)*

Rats were perfused with cold 0.9% saline through the abdominal aorta and the brain

## Effect of eplerenone on cognitive impairment

**Table 1.** Effects of eplerenone on systolic blood pressure in each group

Group	Systolic blood pressure (mmHg)				
	0 week	1 weeks	3 weeks	6 weeks	8 weeks
WKY	118.42 ± 8.56	119.75 ± 7.93	117.50 ± 7.76	119.16 ± 6.82	120.08 ± 7.31
SHR-Veh	189.83 ± 10.39*	192.58 ± 10.15*	191.91 ± 11.02*	194.00 ± 8.87*	193.66 ± 7.10*
SHR-EPL30	190.17 ± 10.53*	186.00 ± 9.80*	183.42 ± 7.48*	184.50 ± 8.21*	184.16 ± 8.28*
SHR-EPL100	190.50 ± 10.26*	182.83 ± 8.00*	176.92 ± 8.24*#,&	171.67 ± 8.80*#,&	169.25 ± 7.90*#,&

Data are expressed as mean ± SD, \*P < 0.05 versus WKY group at the same week, #P < 0.05 versus SHR-Veh group at the same week, &P < 0.05 versus SHR-EPL100 group at 0 week. Significance was determined using one-way analysis of variance (one-way ANOVA) followed by Tukey's post hoc test. SHR: spontaneous hypertensive rat. WKY: Wistar Kyoto rat.

was rapidly isolated. The hippocampal tissue was stripped on ice carefully and completely, then immediately stored at -80°C. The hippocampal tissue was lysed on ice in cold RIPA buffer (AR0102, Boster, China) mixed with 100 mM PMSF (AR1178, Boster, China) and 100 mM protein phosphatase inhibitor (AR1183, Boster, China), then homogenized by ultrasound. The homogenate was centrifuged (12000 rpm, 4°C) for 15 min, and the supernatants were collected. The total protein content of supernatants was determined by a BCA Protein Assay Kit (AR1189, Boster, China) and adjusted to the same level with RIPA and loading buffer, then boiled for 5 min at 95°C. Proteins (20 µg) were separated by SDS-PAGE electrophoresis and transferred to PVDF membranes (0.22 µm, Millipore, Canada). The membranes were blocked in 5% skim milk-TBST for 2 h at room temperature, and incubated with desired primary antibodies overnight at 4°C, then incubated with HRP-conjugated secondary antibodies for another 2 h at room temperature. GAPDH was used as an internal control. The protein bands were developed with ECL luminous kit (AR1171, Boster, China) and detected using Azure c300 Biosystem (c300, Azure Biosystems, USA). The band intensity was analyzed with ImageJ 2.0 (Fuji, Japan).

The primary antibodies used in this study were as follows: anti-mineralocorticoid receptor/NR3C2 (1:1000, PB0817, Boster, China), anti-synaptophysin (1:20000, ab32127, Abcam, UK), anti-PSD95 (1:1000, CY5407, Abways, China), anti-Beclin1 (1:1000, CY5902, Abways, China), anti-LC3B (1:1000, CY5992, Abways, China), anti-P62 (1:5000, CY5546, Abways, China) and anti-GAPDH (1:5000, AP0063, Bioworld, China). The secondary antibody was goat anti rabbit IgG-HRP conjugate (1:5000, RS0002, Immunoway, USA).

### Statistical analysis

All data were presented as mean ± standard deviation (SD). SPSS 21.0 was used for statistical analysis. Before analysis, all data were tested for normal distribution and for homogeneity of variance. Data of the escape latency in the MWM test were analyzed by repeated measures analysis of variance. Other data that fit a normal distribution and with homogeneity of variance were analyzed using one-way analysis of variance (one-way ANOVA) followed by Tukey's post hoc test. Otherwise, data were analyzed by the Kruskal-Wallis non-parametric test with Dunn's multiple comparison test. P < 0.05 was considered significant.

### Results

#### *Eplerenone treatment decreased the blood pressure of SHRs but elevated the serum potassium levels*

Blood pressures of rats were measured before and during the experiment. The blood pressure of the WKY rat is normal and similar to humans under physiologic conditions. As shown in **Table 1**, before the experiment, the systolic blood pressure of rats in the WKY group, SHR-Veh group, SHR-EPL30 group, and SHR-EPL100 group was 118.42 ± 8.56 mmHg, 189.83 ± 10.39 mmHg, 190.17 ± 10.53 mmHg, and 190.58 ± 10.26 mmHg, respectively. Compared to the WKY group, rats in SHR-Veh group, SHR-EPL30 group, and SHR-EPL100 group showed remarkably high blood pressure (P < 0.05). During the experiment, the blood pressure of WKY rats was stable, and the blood pressure in the SHR-Veh group increased slightly without statistical significance. In addition, the blood pressure of rats in SHR-EPL30 was slightly decreased, but there was no significant



## Effect of eplerenone on cognitive impairment

**Table 2.** Effect of eplerenone on serum potassium levels in each group

Group	Serum potassium level (mmol/L)		
	0 week	4 weeks	8 weeks
WKY	5.71 ± 0.90	5.64 ± 0.62	5.89 ± 0.87
SHR-Veh	5.94 ± 1.07	6.03 ± 0.91	6.09 ± 0.72
SHR-EPL30	6.05 ± 0.83	6.16 ± 0.66	6.22 ± 0.80
SHR-EPL100	6.01 ± 0.90	6.34 ± 0.73	6.45 ± 0.94

Data are expressed as mean ± SD, without statistical significance.

difference before and after EPL treatment. More importantly, the systolic blood pressure of rats in the SHR-EPL100 group was 176.92 ± 8.24 mmHg at 3 weeks, and 169.25 ± 7.90 mmHg at 8 weeks after EPL treatment, which was significantly lower than the SHR-Veh group ( $P < 0.05$ ) and before drug treatment ( $P < 0.05$ ).

Considering eplerenone has the side effect of hyperkalemia, serum potassium levels of rats were determined. Before the experiment, there was no difference in serum potassium levels among the four groups (Table 2). By the end of the experiment, serum potassium levels of rats in WKY group, vehicle group, SHR-EPL30 group and SHR-EPL100 group were 5.89 ± 0.87 mmol/L, 6.09 ± 0.72 mmol/L, 6.22 ± 0.80 mmol/L and 6.43 ± 0.94 mmol/L, respectively. Eplerenone treatment showed a tendency to elevate serum potassium levels in SHR, but without statistical significance.

### *Eplerenone treatment improved the short-term working memory of SHRs in Y maze test*

The Y maze alternation test was used to evaluate the short-term working memory of rats. There was no difference in the total arm entries among the 4 groups ( $P > 0.05$ ) (Figure 1A). The percentages of correct spontaneous alternations in WKY, SHR-Veh, SHR-EPL30, and SHR-EPL100 groups were 68.52 ± 2.94%, 55.06 ± 2.90%, 67.04 ± 2.49% and 66.86 ± 3.29%, respectively. Compared to the WKY group, the percentage of correct spontaneous alternations in the SHR-Veh group was decreased ( $P < 0.001$ , Figure 1B), and eplerenone treatment effectively increased the correct spontaneous alternations ( $P < 0.001$ ), but without a difference according to different dosage ( $P > 0.05$ ). These results indicated that the short-term working memory was defective in spontaneous hypertensive rats, and eplere-

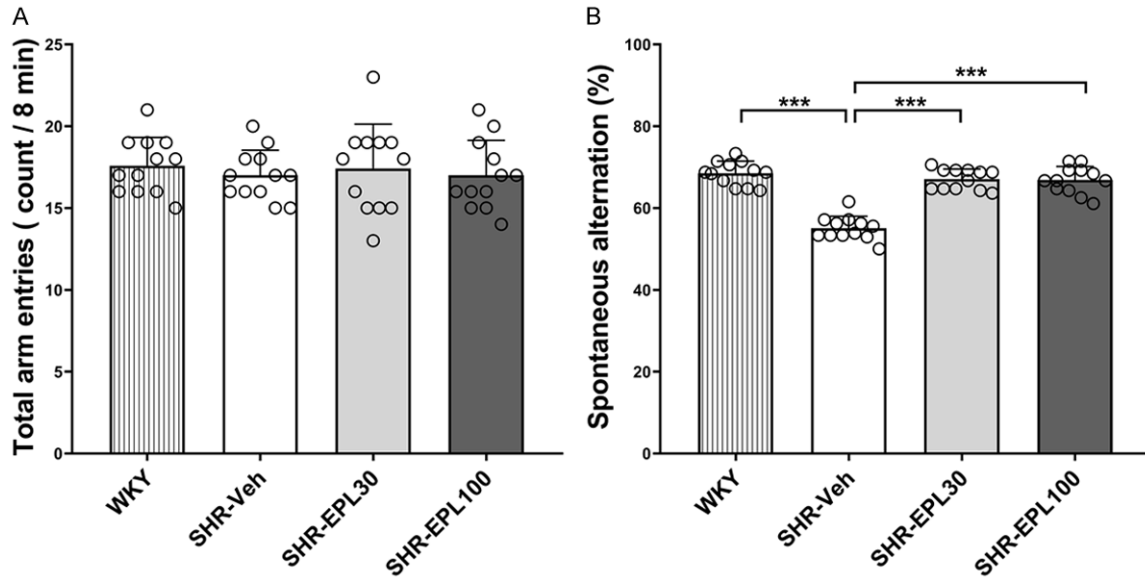
none treatment could improve the impairment in short-term working memory in SHR at a low dosage.

### *Eplerenone treatment improved the long-term spatial learning and memory ability of SHRs in the Morris water maze test*

Morris water maze test was used to evaluate the long-term spatial learning and memory ability of rats. In the spatial navigation training test (Figure 2A, 2B), the average escape latency of rats among the four groups looking for an underwater platform gradually decreased with an increase in training days ( $F(3, 44) = 171.22$ ,  $P < 0.001$ ). On days 3-5, escape latency of the SHR-Veh group was markedly longer than that of the WKY group (day 3:  $P = 0.019$ ; day 4:  $P = 0.005$ ; day 5:  $P < 0.001$ ), and eplerenone treatment significantly decreased escape latency of SHR without a dosage difference (SHR-EPL30 group: day 3:  $P = 0.028$ ; day 4:  $P = 0.010$ ; day 5:  $P < 0.001$ ; SHR-EPL100 group: day 3:  $P = 0.042$ ; day 4:  $P = 0.009$ ; day 5:  $P < 0.001$ ).

In the probe test, the percentage of swimming time in the target quadrant in WKY group, SHR-Veh group, SHR-EPL30 group, and SHR-EPL100 group was 47.90 ± 3.67%, 35.55 ± 3.50%, 47.78 ± 3.57% and 46.56 ± 3.61%, respectively (Figure 2C). The numbers of rats crossing the platform in WKY group, SHR-Veh group, SHR-EPL30 group, and SHR-EPL100 group was 3.83 ± 0.90, 2.33 ± 0.94, 3.75 ± 0.76 and 3.42 ± 1.00, respectively (Figure 2D). Compared to WKY groups, rats in SHR-Veh group showed less swimming time in the target quadrant ( $P = 0.001$ ) and decreased numbers of crossings of the platform ( $P < 0.001$ ). In addition, eplerenone treatment increased the swimming time in the target quadrant ( $P < 0.001$ ) and numbers of crossings of the platform (SHR-EPL30 group:  $P < 0.001$ , SHR-EPL100 group:  $P = 0.018$ ) in SHR. At the same time, the escape latency to reach the visible platform (Figure 2F) and the swimming speed of all rats during the 7 days (Figure 2G) did not show a difference among all groups ( $P > 0.05$ ). The different performances of rats in spatial navigation training and probe tests were unrelated to the vision and swimming ability but related to the damaging effect of hypertension.

## Effect of eplerenone on cognitive impairment



**Figure 1.** Eplerenone improved the short-term working memory impairment of SHRs in a Y maze test. A. There was no significant difference in total arm entries among the four groups. B. Bar graph showing the increased correct spontaneous alternation of SHRs in the SHR-EPL30 group and SHR-EPL100 group. Data are expressed as mean  $\pm$  SD, N = 12. Significance was determined using one-way analysis of variance (one-way ANOVA) followed by Tukey's post hoc test, \*\*\*P < 0.001 vs. SHR-Veh. SHR: spontaneous hypertensive rat. WKY: Wistar Kyoto rat.

*Eplerenone treatment attenuated the neuron damage and increased the expression levels of synaptic proteins in the hippocampus of SHRs*

H&E staining was used to reflect the morphology of hippocampal neurons. CA1, CA3 and DG areas of hippocampus were selected for observation by microscopy (Figure 3). In the SHR-Veh group, the neuronal damage in hippocampal CA1 and CA3 areas was the most obvious, and showed that the number of neuronal cells was decreased, the cells were arranged disorderly and dispersed, and cytoplasmic vacuoles, nuclear pyknosis, and nucleolysis appeared. The pathologic changes of CA1 and CA3 areas in WKY group, SHR-EPL30 group, and SHR-EPL100 group were slight, as hippocampal neurons were arranged orderly, distributed regularly and denser, with distinct layers. The hippocampal neurons were mainly round, with clear cell structure and obvious contrast between nucleus and cytoplasm. Nuclei were clear, bluish, with abundant cytoplasm and few cytoplasmic vacuoles. Although there were disordered and scattered cells and cytoplasmic vacuoles in the DG area of hippocampus in SHR-Veh group, the pathologic changes were mild compared to other groups. These morpho-

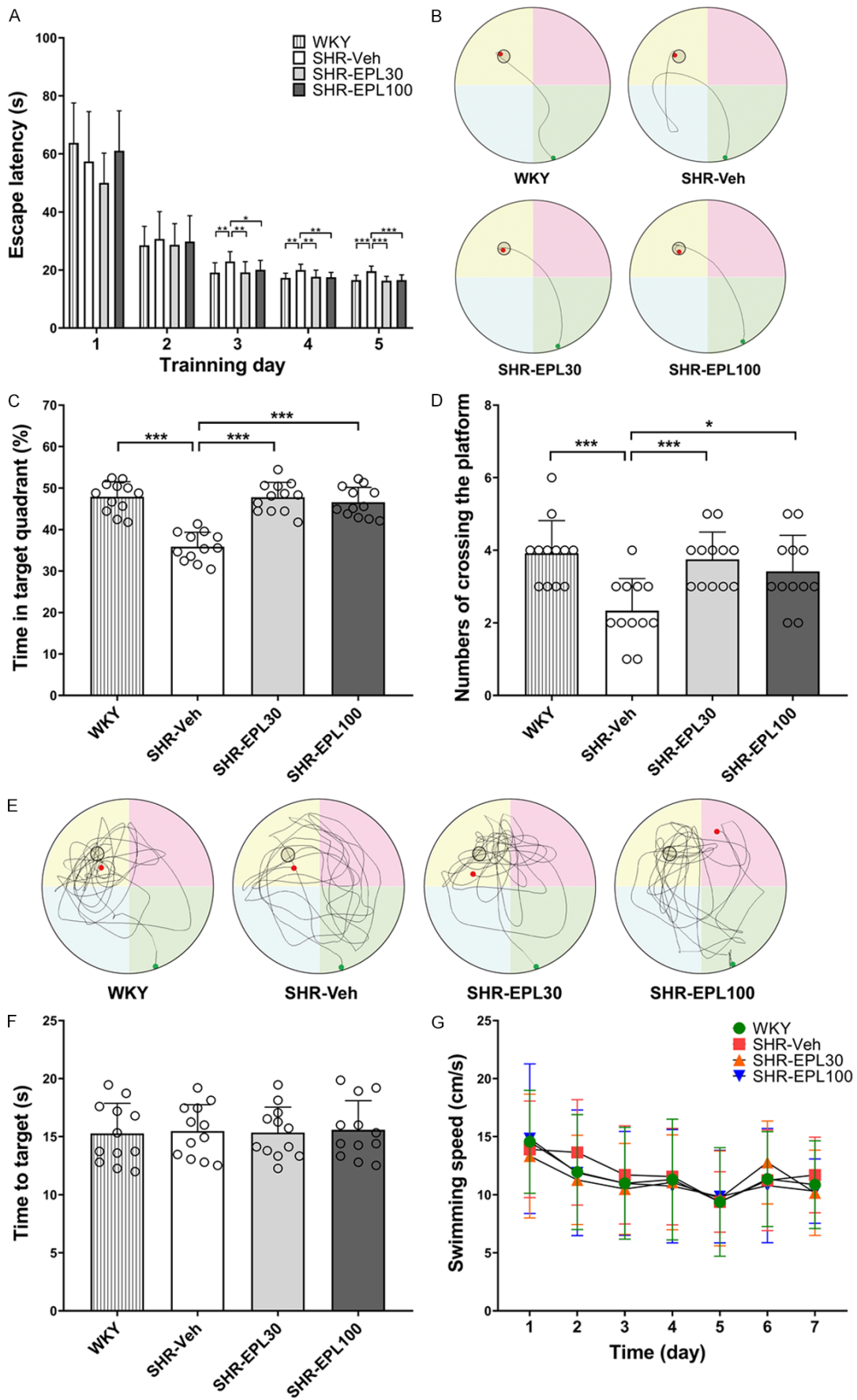
logic results indicated that hypertension caused neuron damage in SHR, and eplerenone treatment attenuated the damage of hippocampal neurons.

Synaptic function is essential for cognitive behavior and synaptic proteins performed important biologic functions. Synaptophysin (SYP) and postsynaptic dense protein 95 (PSD95) were detected to evaluate cognitive function. As shown in Figure 4B, compared to the WKY group, the expression level of PSD95 was significantly decreased in the SHR-Veh group (P = 0.013), and eplerenone treatment increased the expression levels of PSD95 in both the SHR-EPL30 group (P = 0.023) and SHR-EPL100 group (P = 0.039). Although the expression levels of synaptophysin showed no significant difference among the 4 groups, there was an increasing tendency in the SHR-EPL100 group compared to the SHR-Veh group (P = 0.090).

*Eplerenone treatment down-regulated mineralocorticoid receptor expression and increased autophagy in hippocampi of SHRs*

Considering that eplerenone is a MR antagonist, the expression of MR was measured. As

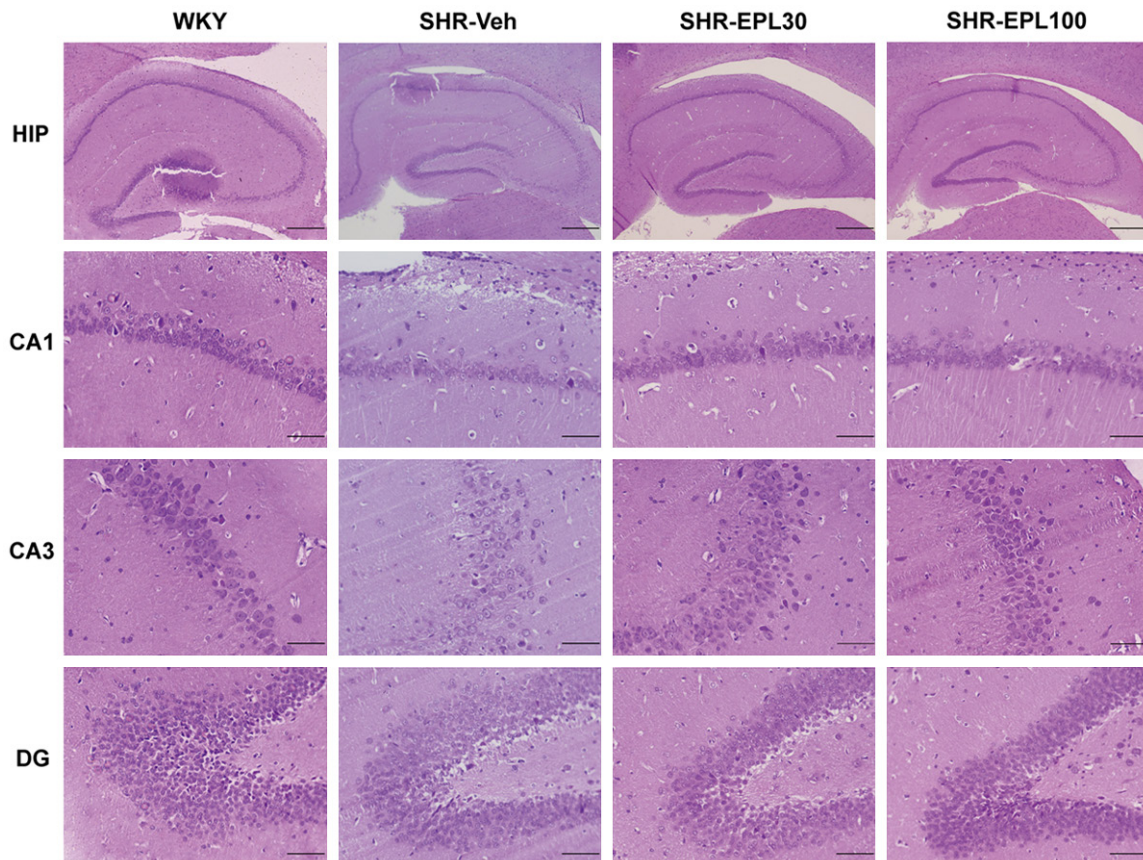
# Effect of eplerenone on cognitive impairment





## Effect of eplerenone on cognitive impairment

**Figure 2.** Eplerenone improves the long-term spatial learning and memory ability of SHRs in Morris water maze (MWM) test. A. Bar graph showing the average escape latencies of rats when searching for the hidden platform in 5 consecutive training days. B. Representative swimming traces of rats on the fifth day of spatial navigation training. The large circle presents the water maze pool, and the small circle represents the hidden platform. The green circle represents the start position and the red dot represents the end position of rats. C. In probe test, bar graph showing the percentage of swimming time of rats spent in the target quadrant. D. In probe test, bar graph showing the numbers of crossing the platform. E. Representative swimming traces of rats in the probe test. F. Bar graph showing the average swimming time of arrival at the visible platform. G. Line graph showing no significant difference in the average swimming speeds among four groups. Data are expressed as mean  $\pm$  SD, N = 12. \*P < 0.05, \*\*P < 0.01, \*\*\*P < 0.001 vs. SHR-Veh.



**Figure 3.** Eplerenone attenuated the neuron damage of SHRs (bar = 200  $\mu$ m). HIP: hippocampus, CA: Cornu Ammonis, DG: dentate gyrus.

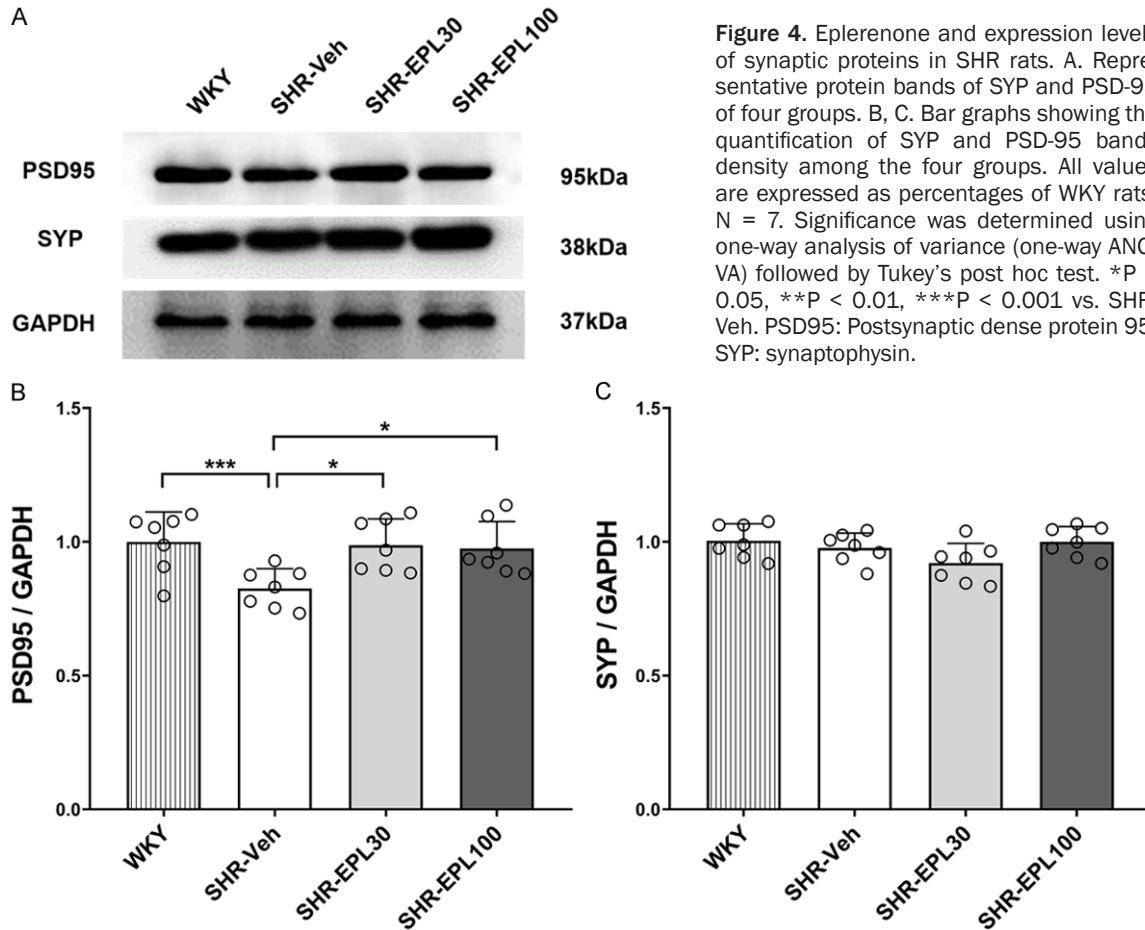
shown in **Figure 5A, 5B**, compared to the WKY group, the expression level of hippocampal MR was increased in the SHR-Veh group (P < 0.001), and eplerenone treatment with a dosage of 100 mg/Kg/d significantly decreased the expression level of hippocampal MR in SHRs (P = 0.036). In addition, compared to the SHR-Veh group, the expression level of hippocampal MR in the SHR-EPL30 group showed a tendency of down-regulation, but without statistical significance (P = 0.275).

Whether MR induces or impairs autophagy in the hippocampus of SHRs is still unclear. Thus,

several autophagy markers were detected in hippocampi of SHR and WKY rats in the present study. Beclin-1 protein was involved in autophagy initiation [25]. As shown in **Figure 5C, 5D**, the expression levels of Beclin-1 protein were decreased in the SHR-Veh group compared to WKY group (P = 0.042). Compared to the SHR-Veh group, eplerenone treatment markedly increased the expression levels of Beclin-1 in both the SHR-EPL30 (P < 0.001) group and SHR-EPL100 group (P = 0.001). Microtubule associated protein 1B light chain 3 (LC3B) is a typical marker during autophagy. LC3B-I transformation to LC3B-II is a symbol



## Effect of eplerenone on cognitive impairment



indicator of autophagosome formation [26]. The LC3B-II/LC3B-I ratios in the WKY group, SHR-Veh group, SHR-EPL30 group, and SHR-EPL100 group were  $0.69 \pm 0.13$ ,  $0.64 \pm 0.17$ ,  $0.87 \pm 0.15$ , and  $0.82 \pm 0.14$ , respectively. The LC3B-II/LC3B-I ratio of the SHR-Veh group was similar to the WKY group, but significantly lower than the SHR-EPL30 group ( $P < 0.001$ ) and SHR-EPL100 group ( $P < 0.001$ ). There was no significant difference between SHR-EPL30 group and SHR-EPL100 group ( $P > 0.05$ , **Figure 5E**). P62 protein degradation occurred at the end of autophagy [27]. P62 expression was significantly increased in the SHR-Veh group ( $P = 0.015$ ) compared to the WKY group. Compared to the SHR-Veh group, the expression levels of P62 were decreased in both the SHR-EPL30 group ( $P = 0.004$ ) and SHR-EPL100 group ( $P = 0.012$ ).

### Discussion

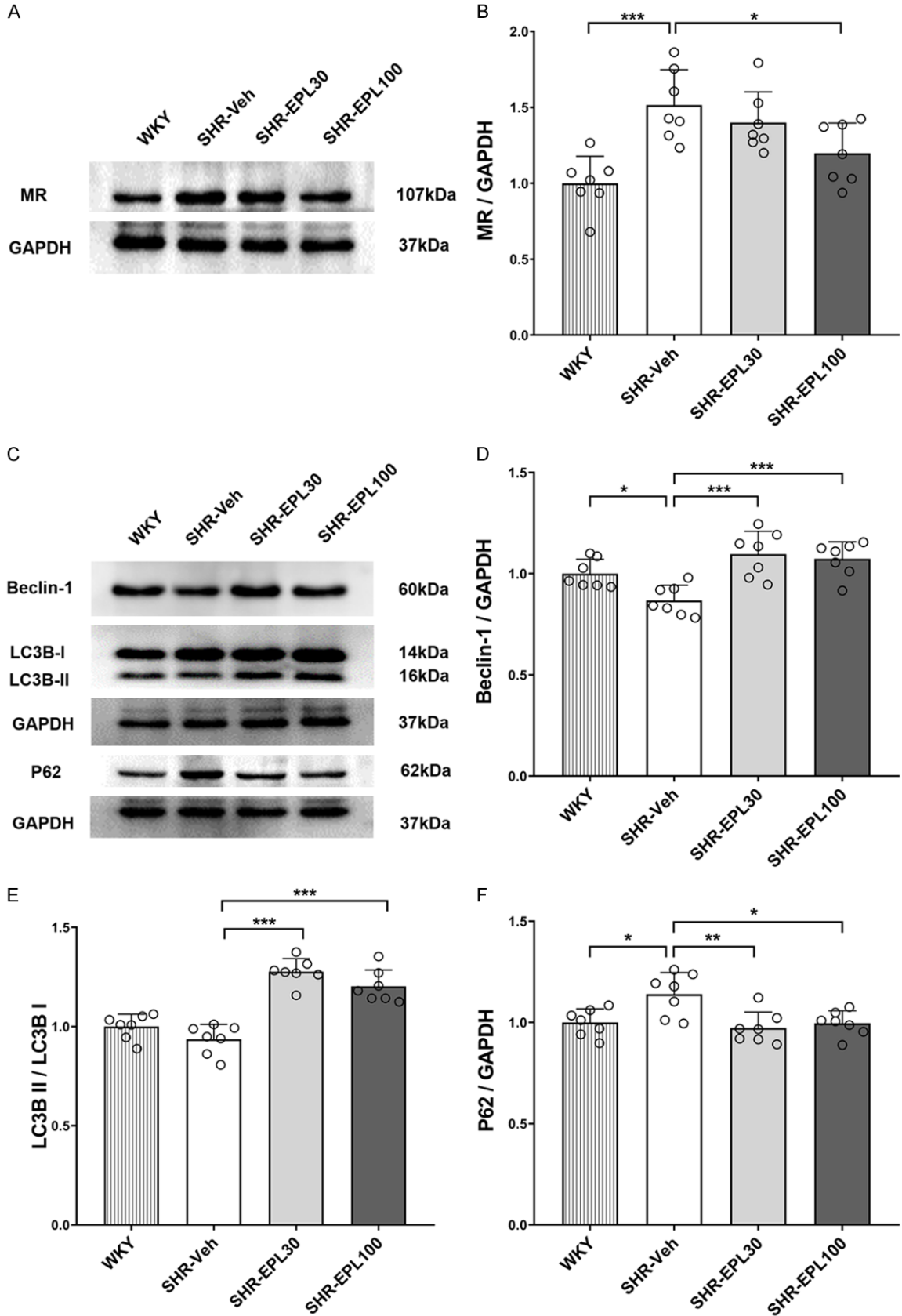
Hypertension is a chronic disease, and its prevalence is gradually increasing among young

individuals [2]. Chronic hypertension can cause functional or organic damage to several organs, including the brain. As hypertension can lead to cognitive impairment, attention should be paid to the protection of neurons along with lowering blood pressure. In this study, the effects of blocking brain MR on cognitive function and the levels of autophagy-related protein expression in SHRs were observed. The results indicated that blocking brain MR may provide a novel way to improve hypertension-related cognitive impairment.

*Eplerenone treatment could attenuate hippocampal neuron damage and increase synaptic protein expression, resulting in improvement of cognitive function in spontaneously hypertensive rats*

The Y maze alternation test and the MWM tests are classical cognitive behavior tests, one for evaluating short-term working memory and another for long-term spatial learning and memory ability. Until now, the effects of eplere-

# Effect of eplerenone on cognitive impairment



## Effect of eplerenone on cognitive impairment

**Figure 5.** Eplerenone downregulates mineralocorticoid receptor expression and activates autophagy function in hippocampi of SHRs. A. Representative protein bands of MR from the same blot of four groups. B. Bar graph showing the quantification of MR among the four groups. C. Representative protein bands of Beclin-1, LC3B, and P62 from the same blot of the groups. D-F. Bar graphs showing the quantification of Beclin-1, P62 band density, and LC3B II/LC3B I ratio among the four groups. All values are expressed as percentage of WKY rats, N = 7. Significance was determined using one-way analysis of variance (one-way ANOVA) followed by Tukey's post hoc test. \*P < 0.05, \*\*P < 0.01, \*\*\*P < 0.001 vs. SHR-Veh. LC3B: Microtubule associated protein 1B light chain 3.

none on cognitive behavior in SHRs were unclear. Some research found that spatial learning and memory were worsened in SHR with age, while the change in short-term working memory was little known [3, 28, 29]. In the present study, the percentage of spontaneous alternations in the SHR-Veh group was lower than that of the WKY group in the Y maze test, indicating that hypertension caused damage to short-term memory in SHR rats. In the Morris water maze test, rats in SHR-Veh group spent more time searching for the underwater platform in the spatial navigation training, but spent less time in the target quadrant in the probe test. This further confirmed the impairments in spatial learning and memory in SHRs, which was consistent with previous reports [29, 30].

Previous research found eplerenone could improve cognitive behavior of Ang-II induced hypertensive mice in the Barnes Maze test [4]. Whether eplerenone improves cognitive behaviors of SHR in the Y maze and MWM tests needs to be explored. In the present study, eplerenone treatment increased the percentage of spontaneous alternation of SHRs in the Y maze test, decreased escape latency, and increased the percentage of swimming time in the target quadrant and the number of crossings of the platform of SHRs in the MWM test. The results of behavioral tests indicated that antagonizing MR by eplerenone could improve the deficits in short-term working memory and long-term memory in SHRs caused by hypertension. More importantly, eplerenone improved cognitive dysfunction in SHRs in both the SHR-EPL30 and SHR-EPL100 groups, and a low dose of eplerenone provided a protective effect.

In the hippocampus, neurons in the CA1 region and dentate gyrus (DG) play an essential role in the learning and acquisition of spatial memories [30, 31]. In the classic trisynaptic pathway, CA3 is required for rapid one-trial contextual learning and for spatial tuning of CA1 cells [32]. Damage to hippocampal CA1 and DG regions in

SHRs has been reported in previous literature [8]. In present study, H&E staining showed obvious damage to neurons in hippocampal CA1 and CA3 regions of the SHR-Veh group. Especially in the CA3 region, the numbers of neurons decreased, the cells were severely dispersed, and shrunken, and nucleolysis appeared. At the same time, scattered cells and cytoplasmic vacuoles were also observed in the DG region of the SHR-Veh group. The morphologic results corresponded to the dysfunction in learning and memory ability in SHRs. Damage in the CA3 region might contribute to impairment of short-term memory. After eplerenone treatment, the damage in hippocampus was attenuated in both SHR-EPL30 group and SHR-EPL100 group, which resulted in dense neurons with clear boundaries and plenty of cytoplasm distributed regularly. Because the CA3 neuron forms a connecting link between the DG and the CA1 region, protection of CA3 neurons is meaningful.

Synaptophysin (SYP), a synaptic vesicle protein, is enriched in axon terminals, and is related to the release of synaptosomes and participates in the transmission of nerve impulses [33]. Postsynaptic dense protein 95 (PSD95) is mainly expressed in the dense region of the postsynaptic membrane, and plays an important role in physiologic processes such as learning, memory and synaptic plasticity [34]. In patients with mild cognitive impairment, gene expression levels of SYP and PSD95 both are both reduced [35]. In SHRs, lower SYP and PSD95 protein levels were associated with worse behavioral performance [28, 29]. In the present study, the expression level of synaptosomes showed no difference among the four groups, but the expression levels of PSD95 decreased significantly in the SHR-Veh group compared to the WKY group, indicating a defect in transduction function of the synapse and synaptic plasticity. Since a decrease of PSD95 was reported to contribute to reduction in the density of dendritic spines and long-term potentiation (LTP) suppression [36, 37], this could

## Effect of eplerenone on cognitive impairment

explain memory impairment and worse behavioral performance observed in SHR. After eplerenone treatment, the decreased expression of PSD95 in SHR was reversed in rats of both the SHR-EPL30 group and SHR-EPL100 group, which was closely related to better performance as shortened escape latency, longer swimming time in the target quadrant, and more correct spontaneous alternations observed in SHR.

*Eplerenone treatment down-regulated MR expression in the hippocampi of SHRs, and enhanced autophagic function was observed*

Recently, mineralocorticoid receptor was reported to be involved in the regulation of neuron excitability and neurogenesis in the hippocampus [38]. Diversities of the mineralocorticoid receptor gene NR3C2 were associated with cognitive function since the participants with AA genotype had higher Mini Mental State Examination (MMSE) scores while with the CG haplotype had decreased memory recall [39]. In the present study, compared to WKY controls, SHRs displayed significantly higher expression levels of hippocampal MR and worse behavioral performance. Eplerenone treatment showed down-regulation of hippocampal MR expression in both the SHR-EPL30 group and SHR-EPL100 group. In the present study, improvement in cognitive behaviors and recovered neuron damage proved the positive effects of blocking hippocampal MR in SHRs. The effect of the mineralocorticoid receptor on intracellular change of neurons is little known.

Autophagy is a physiologic phenomenon that maintains intracellular homeostasis [40]. MR either induced or impaired autophagy in multiple tissue and organs, and both negative effects and protective outcomes were reported. In high-fat-diet-fed mice, blockade of mineralocorticoid receptor impaired autophagy and prevented adipocyte dysfunction [41]. On the other hand, activation of MR could enhance vascular calcification through inhibition of autophagy [42]. There is also a point of view that MR should be concerned with autophagy in the nervous system [43]. However, little is known about the autophagy levels in hippocampus of SHR. Beclin-1, LC3B, and P62 are major autophagy markers. Beclin-1 protein participated in the formation and extension of the autophagosome membrane [25]. Microtubule associated protein 1B light chain 3 (LC3B) is a

structural protein of autophagosome membrane. The increased LC3B-II/LC3B-I ratio indicates enhanced autophagy [44]. P62/SQSTM1 is a key adapter molecule between the autophagosome and lysosome, and is degraded at the end of autophagy [27]. In the present study, compared to normal controls WKY rats were used to reflect the physiologic autophagy level. In the SHR-Veh group, the expression levels of Beclin-1 proteins were decreased and the expression levels of P62 proteins were increased. This indicated an autophagy deficiency in the hippocampi of SHRs. However, eplerenone treatment enhanced autophagy in the SHR-EPL30 group and SHR-EPL100 group as demonstrated by increasing expression of Beclin-1 proteins and the LC3B-II/LC3B-I ratio, suggesting an up-regulation of autophagosome formation. Correspondingly, the reduction of P62 protein indicated that autophagosomes were degraded by lysosomes, furthermore supporting the enhancement of autophagy. Based on the changes in MR, Beclin-1, LC3B and P62 proteins, high expression levels of mineralocorticoid receptor may impair autophagy, while blockade of MR may activate autophagy in the hippocampi of SHRs. Considering that autophagy was involved in amyloid- $\beta$  (A $\beta$ ) clearance and improved cognitive functions in AD model mice [45], it seems that the activation of autophagy in hippocampal neurons of SHRs may promote metabolism of some intracellular substances and play a neuroprotective role. A recent study indicates that UNC-51-like kinase 1 (ULK1) is a regulator of MR activity and is involved in electrolyte homeostasis in renal intercalated cells [46]. ULK1 also has an important role in formation of autophagosomes. Whether MR mediates autophagy through interacting with ULK1 in brain deserves further research. Autophagy has dual effects, so, how to regulate autophagy to achieve the best outcome needs more attention.

*The neuroprotective effect of eplerenone in SHR is independent of lowering blood pressure*

Eplerenone is not the first-line medication for hypertension and its efficacy in lowering blood pressure is dose-dependent [47]. Current studies focus on its effects on organ protection and have proven it can improve ventricular hypertrophy and chronic nephropathy [48, 49]. There are reports that eplerenone treatment



## Effect of eplerenone on cognitive impairment

(50 mg/Kg/d) can attenuate brain lesion of SHR without affecting the blood pressure [8, 50]. In Ang-II induced hypertensive mice, eplerenone could improve cognitive impairment without lowering blood pressure [4]. In the present study, both 30 mg/Kg/d of eplerenone treatment and 100 mg/Kg/d of eplerenone treatment showed protection of cognitive impairment in SHR. Similar to previous findings, 30 mg/Kg/d of eplerenone treatment had little effect on blood pressure. 100 mg/Kg/d of eplerenone treatment lowered blood pressure of SHR, but they were still higher than those of the WKY group. As both dosages of eplerenone treatment enhanced autophagy in hippocampus of SHR, we believe that lowering blood pressure could help protect neurons, but the major protective effect of eplerenone on hippocampal neurons was promoting autophagy and it was independent of lowering blood pressure.

Eplerenone also has the effect of potassium-conserving, and water and sodium excretion. A clinical study has found eplerenone induces minor reductions in serum sodium [51]. Since hyperkalemia can lead to cardiac arrhythmia and bad outcome, monitoring serum potassium is important. Although there was no significant change in blood potassium in this experiment, more studies are needed on how to protect the target organs and minimize the possible adverse consequences of hyperkalemia.

### Limitations

There are some limitations to the present study. First, since there was not a very suitable drug to serve as a positive control in this experiment, more drug dosage groups or drug comparisons (e.g. spironolactone) will be added in future studies to make a more full investigation. Second, the present study primarily shows that eplerenone increases hippocampal autophagy and results in improved cognitive function in SHR, but no autophagy agonists or antagonist were used. In the next study, autophagy regulators will be used to confirm this. Finally, possible signaling pathways were not investigated in the present study and will be studied in depth in the future

### Conclusion

Blockade of MR using eplerenone improves cognitive impairment observed in spontaneously hypertensive rats by promoting autophagy

in hippocampal neurons, increasing expression levels of synaptic proteins, and attenuating neuronal damage. In addition, the protective effects of eplerenone are independent of lowering blood pressure.

### Acknowledgements

The authors gratefully acknowledge the financial support by Key R&D Projects of Shanxi Province (No. 201803D31145), and Shanxi Province Preferential Funding Projects for Returned Overseas (No. 20200331).

### Disclosure of conflict of interest

None.

**Address correspondence to:** Dr. Lei Wang, Department of Geriatrics, Third Hospital of Shanxi Medical University, Shanxi Bethune Hospital, Shanxi Academy of Medical Sciences, No. 99, Longcheng Street, Xiaodian District, Taiyuan 030032, Shanxi, China. Tel: +86-13934226818; E-mail: wang\_leicn2021@163.com

### References

- [1] Wenzel UO, Kemper C and Bode M. The role of complement in arterial hypertension and hypertensive end organ damage. *Br J Pharmacol* 2021; 178: 2849-2862.
- [2] Burnier M and Egan BM. Adherence in hypertension. *Circ Res* 2019; 124: 1124-1140.
- [3] Terry A, Hernandez C, Buccafusco J and Gattu M. Deficits in spatial learning and nicotinic-acetylcholine receptors in older, spontaneously hypertensive rats. *Neuroscience* 2000; 101: 357-368.
- [4] Diaz-Otero JM, Yen TC, Fisher C, Bota D, Jackson WF and Dorrance AM. Mineralocorticoid receptor antagonism improves parenchymal arteriole dilation via a TRPV4-dependent mechanism and prevents cognitive dysfunction in hypertension. *Am J Physiol Heart Circ Physiol* 2018; 315: H1304-H1315.
- [5] Qin J, He Z, Wu L, Wang W, Lin Q, Lin Y and Zheng L. Prevalence of mild cognitive impairment in patients with hypertension: a systematic review and meta-analysis. *Hypertens Res* 2021; 44: 1251-1260.
- [6] Hughes D, Judge C, Murphy R, Loughlin E, Costello M, Whiteley W, Bosch J, O'Donnell MJ and Canavan M. Association of blood pressure lowering with incident dementia or cognitive impairment: a systematic review and meta-analysis. *JAMA* 2020; 323: 1934-1944.
- [7] Heizhati M, Wang L, Li N, Li M, Pan F, Yang Z, Wang Z and Abudereyimu R. Prevalence of

## Effect of eplerenone on cognitive impairment

- mild cognitive impairment is higher in hypertensive population: a cross-sectional study in less developed northwest China. *Medicine (Baltimore)* 2020; 99: e19891.
- [8] Wang X, Zhu Y, Wang S, Wang Z, Sun H, He Y and Yao W. Effects of eplerenone on cerebral aldosterone levels and brain lesions in spontaneously hypertensive rats. *Clin Exp Hypertens* 2020; 42: 531-538.
- [9] Klok M, Alt S, Irurzun Lafitte A, Turner J, Lakke E, Huitinga I, Muller C, Zitman F, de Kloet E and Derijk R. Decreased expression of mineralocorticoid receptor mRNA and its splice variants in postmortem brain regions of patients with major depressive disorder. *J Psychiatr Res* 2011; 45: 871-878.
- [10] Zhang B, Zhu X, Wang L, Hou Z, Hao S, Yang M, Gao F and Liu B. Inadequate expression and activation of mineralocorticoid receptor aggravates spatial memory impairment after traumatic brain injury. *Neuroscience* 2020; 424: 1-11.
- [11] Yagi S, Akaike M, Aihara K, Iwase T, Yoshida S, Sumitomo-Ueda Y, Ikeda Y, Ishikawa K, Matsumoto T and Sata M. High plasma aldosterone concentration is a novel risk factor of cognitive impairment in patients with hypertension. *Hypertens Res* 2011; 34: 74-78.
- [12] Paulsen AJ, Schubert CR, Pinto A, Carlsson CM, Chappell RJ, Fischer ME, Klein BEK, Klein R, Tsai MY and Cruickshanks KJ. Neuroprotective biomarkers and cognitive function in a long-term prospective population-based study of aging US adults. *Alzheimer Dis Assoc Disord* 2020; 34: 31-39.
- [13] Gass P, Kretz O, Wolfer D, Berger S, Tronche F, Reichardt H, Kellendonk C, Lipp H, Schmid W and Schütz G. Genetic disruption of mineralocorticoid receptor leads to impaired neurogenesis and granule cell degeneration in the hippocampus of adult mice. *EMBO Rep* 2000; 1: 447-451.
- [14] Gesmundo I, Villanova T, Gargantini E, Arvat E, Ghigo E and Granata R. The mineralocorticoid agonist fludrocortisone promotes survival and proliferation of adult hippocampal progenitors. *Front Endocrinol* 2016; 7: 66.
- [15] Otte C, Moritz S, Yassouridis A, Koop M, Madrischewski A, Wiedemann K and Kellner M. Blockade of the mineralocorticoid receptor in healthy men: effects on experimentally induced panic symptoms, stress hormones, and cognition. *Neuropsychopharmacology* 2007; 32: 232-238.
- [16] Brocca M, Pietranera L, Meyer M, Lima A, Roig P, de Kloet E and De Nicola A. Mineralocorticoid receptor associates with pro-inflammatory bias in the hippocampus of spontaneously hypertensive rats. *J Neuroendocrinol* 2017; 29: 1-11.
- [17] Shen D, Zhang L, Wei E and Yang Y. Autophagy in synaptic development, function, and pathology. *Neurosci Bull* 2015; 31: 416-426.
- [18] Xu L, Qu C, Qu C, Shen J, Song H, Li Y, Li T, Zheng J and Zhang J. Improvement of autophagy dysfunction as a potential mechanism for environmental enrichment to protect blood-brain barrier in rats with vascular cognitive impairment. *Neurosci Lett* 2020; 739: 135437.
- [19] Chu F, Li K, Li X, Xu L, Huang J and Yang Z. Graphene oxide ameliorates the cognitive impairment through inhibiting PI3K/Akt/mTOR pathway to induce autophagy in AD mouse model. *Neurochem Res* 2021; 46: 309-325.
- [20] Chen K, Zhou X and Sun Z. Haploinsufficiency of *klotho* gene causes arterial stiffening via up-regulation of scleraxis expression and induction of autophagy. *Hypertension* 2015; 66: 1006-1013.
- [21] Li D, Lu Z, Xu Z, Ji J, Zheng Z, Lin S and Yan T. Spironolactone promotes autophagy via inhibiting PI3K/AKT/mTOR signalling pathway and reduce adhesive capacity damage in podocytes under mechanical stress. *Biosci Rep* 2016; 36: e00355.
- [22] Kobayashi N, Yoshida K, Nakano S, Ohno T, Honda T, Tsubokou Y and Matsuoka H. Cardioprotective mechanisms of eplerenone on cardiac performance and remodeling in failing rat hearts. *Hypertension* 2006; 47: 671-679.
- [23] Cleal M, Fontana B, Ranson D, McBride S, Swinny J, Redhead E and Parker M. The free-movement pattern Y-maze: a cross-species measure of working memory and executive function. *Behav Res Methods* 2021; 53: 536-557.
- [24] Vorhees C and Williams M. Morris water maze: procedures for assessing spatial and related forms of learning and memory. *Nat Protoc* 2006; 1: 848-858.
- [25] Xu HD and Qin ZH. Beclin 1, Bcl-2 and autophagy. *Adv Exp Med Biol* 2019; 1206: 109-126.
- [26] Lee YK and Lee JA. Role of the mammalian ATG8/LC3 family in autophagy: differential and compensatory roles in the spatiotemporal regulation of autophagy. *BMB Rep* 2016; 49: 424-430.
- [27] Lamark T, Svenning S and Johansen T. Regulation of selective autophagy: the p62/SQSTM1 paradigm. *Essays Biochem* 2017; 61: 609-624.
- [28] Gao Y, Li W, Liu Y, Wang Y, Zhang J, Li M and Bu M. Effect of telmisartan on preventing learning and memory deficits via peroxisome proliferator-activated receptor- $\gamma$  in vascular dementia spontaneously hypertensive rats. *J Stroke Cerebrovasc Dis* 2018; 27: 277-285.
- [29] Zhang S, Wu D, Xu Q, You L, Zhu J, Wang J, Liu Z, Yang L, Tong M, Hong Q and Chi X. The protective effect and potential mechanism of

## Effect of eplerenone on cognitive impairment

- NRXN1 on learning and memory in ADHD rat models. *Exp Neurol* 2021; 344: 113806.
- [30] Ásgeirsdóttir HN, Cohen SJ and Stackman RW Jr. Object and place information processing by CA1 hippocampal neurons of C57BL/6J mice. *J Neurophysiol* 2020; 123: 1247-1264.
- [31] Hainmueller T and Bartos M. Dentate gyrus circuits for encoding, retrieval and discrimination of episodic memories. *Nat Rev Neurosci* 2020; 21: 153-168.
- [32] Nakashiba T, Young JZ, McHugh TJ, Buhl DL and Tonegawa S. Transgenic inhibition of synaptic transmission reveals role of CA3 output in hippocampal learning. *Science* 2008; 319: 1260-1264.
- [33] Kokotos AC, Harper CB, Marland J, Smillie KJ and Gordon SL. Synaptophysin sustains pre-synaptic performance by preserving vesicular synaptobrevin-II levels. *J Neurochem* 2019; 151: 28-37.
- [34] Matt L, Kim K, Chowdhury D and Hell JW. Role of palmitoylation of postsynaptic proteins in promoting synaptic plasticity. *Front Mol Neurosci* 2019; 12: 8.
- [35] Counts SE, Alldred MJ, Che S, Ginsberg SD and Mufson EJ. Synaptic gene dysregulation within hippocampal CA1 pyramidal neurons in mild cognitive impairment. *Neuropharmacology* 2014; 79: 172-179.
- [36] Mardones MD, Jorquera PV, Herrera-Soto A, Ampuero E, Bustos FJ, van Zundert B and Varela-Nallar L. PSD95 regulates morphological development of adult-born granule neurons in the mouse hippocampus. *J Chem Neuroanat* 2019; 98: 117-123.
- [37] Wang C, Gao WR, Yin J, Wang ZJ, Qi JS, Cai HY and Wu MN. Chronic sleep deprivation exacerbates cognitive and synaptic plasticity impairments in APP/PS1 transgenic mice. *Behav Brain Res* 2021; 412: 113400.
- [38] Le Menuet D and Lombès M. The neuronal mineralocorticoid receptor: from cell survival to neurogenesis. *Steroids* 2014; 91: 11-19.
- [39] Terock J, Van der Auwera S, Janowitz D, Wittfeld K, Teumer A and Grabe HJ. Functional polymorphisms of the mineralocorticoid receptor gene NR3C2 are associated with diminished memory decline: results from a longitudinal general-population study. *Mol Genet Genomic Med* 2020; 8: e1345.
- [40] Galluzzi L and Green DR. Autophagy-independent functions of the autophagy machinery. *Cell* 2019; 177: 1682-1699.
- [41] Armani A, Cinti F, Marzolla V, Morgan J, Cranstom G, Antelmi A, Carpinelli G, Canese R, Pagotto U, Quarta C, Malorni W, Matarrese P, Marconi M, Fabbri A, Rosano G, Cinti S, Young M and Caprio M. Mineralocorticoid receptor antagonism induces browning of white adipose tissue through impairment of autophagy and prevents adipocyte dysfunction in high-fat-diet-fed mice. *FASEB J* 2014; 28: 3745-3757.
- [42] Gao J, He W, Xie C, Gao M, Feng L, Liu Z, Wang J, Huang H and Liu P. Aldosterone enhances high phosphate-induced vascular calcification through inhibition of AMPK-mediated autophagy. *J Cell Mol Med* 2020; 24: 13648-13659.
- [43] Agnieszka W and Ma Gorzata K. Steroid and xenobiotic receptor signalling in apoptosis and autophagy of the nervous system. *Int J Mol Sci* 2017; 18: 2394.
- [44] Cerulli RA, Shehaj L, Brown H, Pace J, Mei Y and Kritzer JA. Stapled peptide inhibitors of autophagy adapter LC3B. *Chembiochem* 2020; 21: 2777-2785.
- [45] Luo R, Su LY, Li G, Yang J, Liu Q, Yang LX, Zhang DF, Zhou H, Xu M, Fan Y, Li J and Yao YG. Activation of PPARA-mediated autophagy reduces Alzheimer disease-like pathology and cognitive decline in a murine model. *Autophagy* 2020; 16: 52-69.
- [46] Shibata S, Ishizawa K, Wang Q, Xu N, Fujita T, Uchida S and Lifton RP. ULK1 phosphorylates and regulates mineralocorticoid receptor. *Cell Rep* 2018; 24: 569-576.
- [47] Tam TS, Wu MH, Masson SC, Tsang MP, Stabler SN, Kinkade A, Tung A and Tejani AM. Eplerenone for hypertension. *Cochrane Database Syst Rev* 2017; 2: Cd008996.
- [48] Schneider A, Schwab J, Karg MV, Kalizki T, Reinold A, Schneider MP, Schmieder RE and Schmidt BM. Low-dose eplerenone decreases left ventricular mass in treatment-resistant hypertension. *J Hypertens* 2017; 35: 1086-1092.
- [49] Chung EY, Ruospo M, Natale P, Bolignano D, Navaneethan SD, Palmer SC and Strippoli GF. Aldosterone antagonists in addition to renin angiotensin system antagonists for preventing the progression of chronic kidney disease. *Cochrane Database Syst Rev* 2020; 10: Cd007004.
- [50] Sepehrdad R, Chander PN, Oruene A, Rosenfeld L, Levine S and Stier CT. Amiloride reduces stroke and renal injury in stroke-prone hypertensive rats. *Am J Hypertens* 2003; 16: 312-318.
- [51] Martens P, Ferreira JP, Vincent J, Abreu P, Busse M, Mullens W, Tang WWH, Böhm M, Pitt B, Zannad F and Rossignol P. Serum sodium and eplerenone use in patients with a myocardial infarction and left ventricular dysfunction or heart failure: insights from the EPHEBUS trial. *Clin Res Cardiol* 2021; 4: 1-13.