Hypothalamic paraventricular nucleus hydrogen sulfide exerts antihypertensive effects in spontaneously hypertensive rats by attenuating oxidative stress via the Nrf2 pathway

Wen-Jie Xia
Xi'an Jiaotong University School of Basic Medical Sciences
Xiao-Jing Yu
Xi'an Jiaotong University School of Basic Medical Sciences
Kai-Li Liu
Xi'an Jiaotong University School of Basic Medical Sciences
Xiao-Min Wang
Xi'an Jiaotong University School of Basic Medical Sciences
Yu Yang
Xi'an Jiaotong University School of Basic Medical Sciences
Tingting Meng
Xi'an Jiaotong University School of Basic Medical Sciences
Jin-An Qiao
Xi'an Jiaotong University School of Basic Medical Sciences
Nianping Zhang
Shanxi Datong University School of Medicine
Yao-Jun Sun
Shanxi Medical University, Shanxi Medical University
Yu-Ming Kang (ykang@mail.xjtu.edu.cn)
Xi'an Jiaotong University School of Basic Medical Sciences

Research Article

Keywords: hydrogen sulfide, hypertension, paraventricular nucleus, Nrf2, oxidative stress

Posted Date: August 24th, 2022

DOI: https://doi.org/10.21203/rs.3.rs-1974695/v1

Abstract

Hydrogen sulfide (H_2S) is widely distributed throughout the nervous system with various antioxidant and anti-inflammatory properties. Increased reactive oxygen species and inflammation in the hypothalamic paraventricular nucleus (PVN) are involved in the pathophysiology of hypertension. But it is unclear how H_2S in PVN affects hypertension.

Our study used spontaneously hypertensive rats (SHR) and control Wistar Kyoto (WKY) rats, microinjected with AAV-CBS (cystathionine beta-synthase overexpression) or AAV-ZsGreeen in the bilateral PVN; or simultaneously injected with virus-carrying nuclear factor erythroid 2-related factor 2 (Nrf2)-shRNA. We found that AAV-CBS increased H_2S in the PVN, and that blood pressure, neuronal activation, oxidative stress, and inflammation of PVN were all substantially reduced. In addition, PVN endogenous H_2S activated Nrf2 and corrected the PVN's unbalanced of excitatory and inhibitory neurotransmitters. However, Nrf2 knockdown in the PVN was similarly observed to abolish the beneficial effect of H_2S on hypertension. These results suggest that PVN endogenous H_2S can ameliorate hypertension through Nrf2-mediated antioxidant and anti-inflammatory effects.

Introduction

In the formation and progression of hypertension (HTN), the central nervous system's regulation is essential. Resistant HTN is caused in part by abnormal autonomic mechanisms, which are characterized by sympathetic nervous system hyperactivity and defective cardiac vagal control. The hypothalamic paraventricular nucleus (PVN) regulates respiration, blood pressure (BP), and cardiovascular activity as an integrative element of the neuroendocrine and autonomic nervous systems (Rostami & Hatam, 2022; Xia et al., 2021; Yu et al., 2022). We and other research teams have previously demonstrated that reactive oxygen species (ROS) buildup and an unbalanced ratio of anti- to pro-inflammatory cytokines (PICs) in the PVN were shown to be associated with high blood pressure in hypertensive rats (Gao et al., 2021; Kang et al., 2019; Yu et al., 2021). The elevated sympathetic nervous system excitability may be significantly influenced by high ROS and PICs levels in the PVN. Treatment with antioxidants or anti-inflammatory drugs in the PVN has been proved to be effective in improving blood pressure. Therefore, reducing the amounts of PICs, ROS, and NADPH oxidase in the PVN may be a useful method for lowering blood pressure. In addition, in different hypertension models, the level of neurotransmitter imbalance in the PVN is a substantial contributor to sympathetic output (Yu et al., 2021).

Hydrogen sulfide (H_2S) is a gas transmitter created by three enzymes in the cytoplasm or mitochondria from homocysteine (Hcy), L-cysteine (Cys), or β-mercaptopyruvate by three enzymes in the cytoplasm or mitochondria. Cystathionine beta-synthase (CBS) is the main enzyme that produces H_2S in the central nervous system (CNS), cystathionine gamma-lyase (CSE) is mainly found in peripheral and cardiovascular tissues, while 3-mercaptopyruvate sulfur-transferase (3-MST) is mostly found in mitochondria.

Studies (Li et al., 2022; Xu et al., 2022) have demonstrated that endogenous H_2S has anti-inflammatory properties. H_2S has been demonstrated to inhibit monocyte chemotaxis and decrease the synthesis of PICs like tumor necrosis factor-α (TNF-α), and interleukin-6 (IL-6) in a variety of in vitro and in vivo investigations. Furthermore, H_2S ameliorated paraquat-induced acute liver damage by increasing antioxidant property, modulating mitochondrial function, and reducing NLRP3 activation of the inflammasome induced by ROS (Liu et al., 2020). H_2S is clearly effective at reducing inflammation and acting as an antioxidant.

Numerous studies have implicated decreased endogenous H_2S production or an enzyme deficiency in the development of hypertension. Spontaneously hypertensive rats (SHR) showed a decrease in circulating H_2S levels; early administration of H_2S, D-, or L-cysteine could lower blood pressure (BP) (Hsu et al., 2018). In mice, deletion of CSE lowered serum levels of H_2S, the degree of endothelial vasodilation, and H_2S concentrations in various tissues, and these mutant mice had severe hypertension (Yang et al., 2008). Most noteworthy, H_2S can play a role in the CNS. NaHS microinjections into the rostroventrolateral medulla (RVLM) reduced ROS and hence lowered BP in spontaneously hypertensive rats (Yu et al., 2015). Chronic NaHS intracerebroventricular (ICV) infusion can lower blood pressure, enhance autonomic activity, and have an impact on PVN microglia (Donertas Ayaz et al., 2021). In high salt-induced hypertension, our group has discovered that chronic infusion of GYY4137, an H_2S donor, in bilateral PVN for four weeks, may lower sympathetic activity and the hypertensive response, in part because ROS and PICs levels inside the PVN are reduced (Liang et al., 2017).

Nuclear factor-erythroid 2-related factor 2 (Nrf2) promotes the transcription of cell-related protective genes by combining with antioxidant response elements (ARE), and it is crucial for maintaining cellular antioxidants and preventing diseases associated with them. It can also adjust ROS production via controlling NADPH oxidase and mitochondria. Studies have shown that curcumin can improve hypertension by inducing Nrf2 into the nucleus (Tapia et al., 2012); resveratrol can reduce the renal inflammatory response in SHR by activating the Nrf2 pathway (Javkhedkar et al., 2015); and during hypertension, the RVLM mediates abnormal mitochondrial production (Wu et al., 2016). Recent research has demonstrated that a particular Nrf2 gene deletion in the RVLM can reduce the expression of antioxidant enzymes, boost sympathetic nerve activity (SNA), and raise BP (Gao et al., 2017). According to our group's research, the PVN's oxidative stress and inflammation can be inhibited by activating the Nrf2 pathway, which in turn lowers blood pressure (Bai et al., 2017; Sun et al., 2017).

The purpose of this study was to elucidate the specific mechanism by which hydrogen sulfide activation of the Nrf2 pathway in the PVN reduces oxidative stress and inflammatory responses in the PVN during hypertension and subsequently attenuates sympathetic activity and blood pressure.

Materials And Methods

Animals

This experiment complied with the NIH Guide for the Care and Use of Laboratory Animals and was approved by the Xi'an Jiaotong University Ethics Committee of Laboratory Animals. Charles River Laboratories provided SHR and Wistar Kyoto (WKY) male rats weighing 220-250g. Rats were kept in a room with a controlled temperature (23±2°C) and lighting (12-h/12-h light-dark cycle) along with a regular diet and tap water.

Adenovirus-Associated Virus (AAV) Preparation

Experiment 1: Hanbio Biotechnology Co. Ltd. (Shanghai, China) provided the control vectors (HBAAV2/9-ZsGreen) and the plasmid vector (pHBAAV-CMV-MCS-3flag-T2A-ZsGreen) carrying the mRNA of Rattus norvegicus CBS targeting sequence (Transcript: NM 012522.2) (contract number: KWC20200630ZY-AAV01).

Experiment 2: Hanbio Biotechnology Co. Ltd provided the plasmid vector (pHBAAV-U6-MCS-CMV-EGFP) containing the Nrf2-small hairpin RNA (AAV-Nrf2 shRNA) and control vectors (AAV-EGFP NC) (contract number: HH20210514ZY-AAV01).

The AAV has a titer of 1×10^{12} μg/ml, and was sub-packaged (20 μl/tube) and store at -80°C. And the vectors needed to be mixed 1:1 before injection in experiment 2.

AAV Injection

AAV was infused into the PVN as previously mentioned (Su et al., 2021). Briefly, rats were fixed with the head on the brain stereotaxic equipment after intraperitoneal ketamine (80 mg/kg) and xylazine (10 mg/kg) anesthesia. AAV was contained in a 5 μl microinjector that was attached to an infusion pump. Following this, within 10 minutes, 1 μl (experiment 1) or 2 μl (experiment 2) of AAV were infused bilaterally into the PVN (the location referenced to Paxinos and Watson rat brain atlases the Paxinos and Watson rat brain atlas). After the injection, the microinjector was left for ten minutes and then pulled out.

General experimental protocol

Two weeks were spent acclimating the rats to their surroundings prior to the start of the experiment. Each group of rats was randomly assigned: experiment 1 (n = 10 each group): (i) WKY + AAV- ZsGreen, (ii) WKY + AAV-CBS, (iii) SHR + AAV- ZsGreen, (iv) SHR + AAV-CBS, experiment 2 (n = 10 each group): (i) SHR + AAV- ZsGreen + NC, (ii) SHR + AAV- ZsGreen + Nrf2 shRNA, (iii) SHR + AAV-CBS+ Nrf2 shRNA, (iv) SHR + AAV-CBS + NC. Four weeks after the PVN injection, the trial came to an end. Plasma samples were obtained as described previously, and PVN was isolated from fresh rat brain tissue. another portion of fresh rat brain tissue was stored in 4% paraformaldehyde for three days, then dehydrated in 30% sucrose, and finally embedded in OCT. To prepare samples for subsequent analysis, they were all stored at -80 °C.

Blood pressure measurements

We measured BP every 4 days between 8:00 and 11:00 AM by tail-cuff plethysmography as previously described (Xia et al., 2021). Additionally, anesthesia-induced rats' left carotid arteries were intubated with polyethylene catheters at the conclusion of the experiment in order to assess mean arterial blood pressure (MAP) as previously mentioned. Within 30 minutes, MAP and heart rate (HR) data were gathered and averaged.

H_2S level in the PVN

PVN was isolated from rat brain, and the H_2S level was determined using an H_2S concentration determining kit (Solarbio Science & Technology Co., Ltd. Beijing, China) as per the instruction.

Immunofluorescence and immunohistochemistry

OCT-embedded brain tissue was cut with a thickness of 18um from bregma -0.92 to -2.12mm in a frozen microtome (Leica, CM1860). As previously mentioned, the PVN immunohistochemical and immunofluorescence staining was carried out. The primary antibodies used in the study consisted of the following: rabbit anti-Nrf2 (Abcam), mouse anti-CBS, rabbit anti-Fra-Like, rabbit anti-gp91^{phox}, mouse anti-p47^{phox}, mouse anti-IL-10, rabbit anti-GAD67 (Santa Cruz), rabbit anti-TH (Millipore Sigma), rabbit anti-IL-1β, rabbit anti-HO-1 (Bioss). Finally, fluorescence microscope images of slices stained with immunofluorescence were obtained (Nikon Eclipse, 80i, Japan).

In immunohistochemistry, slices were treated for 1 hour in a blocking solution with a secondary antibody from Abcam. Next, we detected the horseradish peroxidase response using the 3,3-diaminobenzidine (DAB) kit (beyotime) to detect the horseradish peroxidase reaction. With the microscope, sections were captured in photographs.

Dihydroethidium staining

To determine whether superoxide was forming in the PVN, dihydroethidium (DHE) staining was used, and the procedure was carried out as previously described (Su et al., 2016). Finally, sections were imaged by a Nikon fluorescence microscope.

Western blotting

As previously mentioned, the Western blotting methodology was followed (Xia et al., 2021). The primary antibodies used in the study consisted of the following: rabbit anti-Nrf2, rabbit anti-SOD1, mouse anti-IL-6, rabbit anti-HO-1 (Abcam), mouse anti-CBS, rabbit anti-Fra-Like, rabbit anti-gp91^{phox}, mouse anti-p47^{phox}, mouse anti-TNF-α, mouse anti-IL-10, mouse anti-TH, rabbit anti-GAD67 (Santa Cruz), rabbit anti-IL-1β (Bioss).

ELISA

According to the manufacturer's instructions, a commercial ELISA kit (Abnova, Taiwan) was used to test the levels of norepinephrine (NE), IL-1β, and IL-6 in plasma.

Statistical analysis

Data from the group were presented as mean ± SEM, with a Probability (*P*) value <0.05 denoting significance. The SBP values were analyzed using repeated-measures ANOVA. Other parameters in experiment 1 were analyzed by two-way ANOVA with Tukey's post hoc test. Other parameters in experiment 2 were analyzed by one-way ANOVA and Tukey's post hoc test. The data was analyzed and graphics were produced using GraphPad Prism (Version 7.0, La Jolla, CA, USA).

Results

AAV-CBS increased CBS expression and H_2S level in the PVN

As shown in Figure 1A, after injection of AAV into PVN, ZsGreen was strongly expressed in the PVN but not in the supraoptic nucleus (SON) or the subfornical organ (SFO). Additionally, SHR showed decreased CBS expression (Figures 1B and C) and H_2S levels (Figure 1D) in their PVN compared to control rats. Administration of AAV-CBS to PVN could significantly increase PVN expression of CBS and H_2S levels in SHR.

Endogenous H_2S in the PVN lowered BP and HR of SHR

In our investigation, SBP was measured using a tail-cuff plethysmograph. As shown in Figure 2A, SHR had a significant increase in blood pressure compared with WKY rats (SHR + AAV- ZsGreen: 186±3 mmHg vs WKY+ AAV- ZsGreen: 125±4 mmHg, P<0.05, HR: SHR + AAV- ZsGreen: 186±3 mmHg vs WKY+ AAV- ZsGreen: 125±4 mmHg, P<0.05). From day 12, the blood pressure in the SHR + AAV- CBS rats gradually dropped and stayed lower than in the SHR + AAV- ZsGreen group by the end of our study (SHR + AAV- CBS: 155±5 mmHg vs SHR + AAV- ZsGreen: 186±3 mmHg, P<0.05). These SBP data were supported by MAP records (Figure 2B). Even though endogenous H_2S in PVN also decreased SHR's heart rate (Figure 2C), the impact was insignificant. Additionally, we discovered that H_2S had no discernible impact on WKY rats' BP and HR.

Endogenous H_2S in the PVN decreased the expression of Fra-LI and plasma levels of NE of SHR

Plasma NE levels were an indirect predictor of sympathetic activity, and additionally, we examined Fra-LI expression in the PVN in order to evaluate the impact of endogenous H_2S on neuronal activity. As shown in Figure 2, both Fra-LI expression in the PVN (Figure 2D) and plasma NE levels (Figure 2E) were much higher in SHR than those in WKY rats. Increased endogenous H_2S in the PVN significantly reduced PVN Fra-LI expression and plasma NE concentration of SHR. Additionally, we discovered that in WKY rats, H_2S had no discernible impact on Fra-LI expression or plasma NE levels.

Endogenous H_2S in the PVN increased Nrf2 expression of SHR

According to immunofluorescence and western blotting analyses, compared to the WKY rats, Nrf2 expression in the PVN of SHR was lower in SHR, and Nrf2 expression was raised by an increase in endogenous H_2S in SHR but not in WKY rats (Figures 3A and B).

Endogenous H_2S in the PVN attenuated oxidative stress of SHR

We first detected ROS changes in the PVN using dihydroethidium (DHE) staining. Endogenous H_2S in the PVN significantly decreased the increase of ROS in SHR but not in WKY rats as shown in Figure 3C.

The primary functional subunit of NADPH oxidase (NOX) is $gp91^{phox}$. It and $p22^{phox}$ subunit are located on the plasma membrane. After being stimulated, the two combine to form a NOX complex. The $p47^{phox}$ subunit in the cytoplasm interacts with the $p22^{phox}$ subunit after phosphorylation, resulting in a conformational change. $P47^{phox}$ binds to other cytoplasmic subunits ($p67^{phox}$ and $p40^{phox}$) to form the activated NOX. Their levels have been measured in numerous earlier investigations to represent NOX activity (Bai et al., 2017). Results revealed that compared to the control rats, the number of $gp91^{phox}$ (Figure 3D) and $p47^{phox}$-positive neurons (Figures 3E), as well as their protein levels (Figure 3F) in the PVN of SHR rats, were significantly higher. After microinjection of AAV-CBS into the PVN bilaterally, the number of $gp91^{phox}$ and $p47^{phox}$-positive neurons, as well as their protein levels, were decreased.

Superoxide dismutase (SOD), an antioxidant metalloenzyme, can catalyze the dismutation reaction of superoxide anion radicals to produce O_2 and H_2O_2 in living things. SOD is essential for maintaining the proper balance of oxidation and antioxidants in the body (Su et al., 2021). SOD1 mainly exists in the cytoplasm of eukaryotic cells and is considered to be the most widely distributed one among the primitive biological groups. As shown in Figure 3F, compared to the WKY rats, SHR had SOD1 expression was lower in the PVN, but endogenous H_2S in the PVN increased SOD1 expression in SHR.

Endogenous H_2S in the PVN reduced PICs of SHR

Increased neuroinflammation in autonomic brain regions has been identified as strongly associated with hypertension. Therefore, we evaluated the changes of IL-1β, TNF-α, IL-6, and IL-10 in the PVN. Compared to the WKY + AAV- ZsGreen group, significantly increased numbers of IL-1β-positive neurons (Figure 4A) and decreased numbers of IL-10-positive neurons (Figure4B) were observed in the PVN of the SHR + AAV-ZsGreen group. Whereas, in SHR but not WKY rats, endogenous H_2S in the PVN dramatically reduced the number of IL-1β-positive neurons and increased the number of IL-10-positive neurons. In addition, western blotting results revealed that the protein levels of TNF-α, IL-1β, and IL-6 in the PVN of SHR were significantly higher than those of WKY rats, while the protein level of IL-10 was significantly lower than that of WKY rats (Figure 4C). In SHR, endogenous H_2S in the PVN dramatically boosted the level of IL-10 and lowered the level of TNF-α, IL-1β, and IL-6, but not in WKY rats. Additionally, we used an ELISA technique to measure IL-1β and IL-6 expression to ascertain the impact of endogenous H_2S on plasma

levels of pro-inflammation cytokines. Plasma levels of IL-1β (Figure S1A) and IL-6 (Figure S2B) were higher in the SHR+AAV-ZsGreen group than in the WKY+AAV-ZsGreen group, and these changes were significantly reduced in the group that received AAV-CBS PVN microinjections. Additionally, we discovered that in WKY rats, H_2S had no discernible impact on the plasma level of pro-inflammatory cytokines.

In conclusion, the current results demonstrate that increased endogenous H_2S in the PVN could restore the balance of anti- and pro-inflammatory cytokines and reduces the plasma level of inflammatory cytokines in SHR.

Endogenous H_2S in the PVN restored neurotransmitter imbalance of SHR

In the PVN, GABA is a significant inhibitory neurotransmitter, and norepinephrine (NE) is a significant excitatory neurotransmitter. They are closely related to how sympathetic nerve activity is controlled in hypertension. A crucial enzyme in the manufacture of catecholamines (including NE, epinephrine, and dopamine) is tyrosine hydroxylase (TH). The enzyme glutamate decarboxylase (GAD) 67 is essential for the synthesis of GABA. The levels of NE and GABA may be indirectly reflected in the expression of TH and GAD67.

Immunofluorescence and immunohistochemistry results indicated that compared to the WKY group, SHR had significantly more TH-positive neurons (Figure 4E) and significantly fewer GAD67-positive neurons (Figure 4D) in the PVN. Endogenous H_2S in the PVN markedly enhanced GAD67 expression while decreasing TH expression. The results of western blotting were consistent with immunofluorescence and immunohistochemistry studies (Figure 4F). Additionally, we discovered that in WKY rats, H_2S had no discernible impact on TH or GAD67.

Microinjection of Nrf2 shRNA into PVN decreased the expression of Nrf2

Next, we observed the expression level of Nrf2 after injecting AAV-Nrf2 shRNA into PVN to assess the consequences of Nrf2 silencing. Western blotting results revealed that SHR treated with AAV-Nrf2 shRNA had considerably lower levels of Nrf2 protein in their PVN than SHR treated with AAV-EGFP NC (Figure 5A).

The antihypertensive impact of endogenous H_2S in SHR is eliminated by PVN knockdown Nrf2

Next, we assessed whether endogenous H_2S in the PVN ameliorates hypertension through the Nrf2 pathway. As demonstrated in Figure 5B, compared to the AAV-Nrf2 shRNA group, PVN endogenous H_2S lowered blood pressure in the SHR+AAV-EGFP NC group. The findings showed that the hypotensive impact of endogenous H_2S might be inhibited by decreasing Nrf2 expression in PVN.

The Nrf2-dependent antioxidant response of endogenous H_2S in the PVN of SHR is eliminated by PVN knockdown Nrf2

The heme oxygenase-1 (HO-1) is a powerful antioxidant, antioxidant, and Nrf2 can directly regulate the activity of the HO-1 promoter. The results showed that endogenous H_2S in the PVN increased the HO-1 positive neuron number (Figure 5C) and the expression level of HO-1 protein (Figure 5D) in SHR, but in the AAV-Nrf2 shRNA group, the effect of H_2S was eliminated. These data suggest that the Nrf2/HO-1 pathway is the mechanism by which PVN endogenous H_2S acts.

The effect of PVN endogenous H_2S on oxidative stress of SHR is abrogated by PVN knockdown Nrf2

As shown in Figure 8, we found that endogenous H_2S in the PVN reduced ROS formation (Figure 6A) and the expression of $gp91^{phox}$ (Figures 6B and C) and $p47^{phox}$ (Figure 6C), increased SOD1 (Figure 6C) protein expression level in SHR + AAV EGFP NC group. These changes were not present in the SHR + Nrf2 shRNA group. These findings imply that Nrf2 knockdown can completely reverse the effects of PVN endogenous H_2S on oxidative stress and antioxidant capacity.

The effect of endogenous H_2S on inflammation and neurotransmitter in the PVN of SHR is abrogated by PVN knockdown Nrf2

The result presented in Figures S2 and S3 demonstrated that PVN bilateral microinjection of AAV-CBS reduced the level of TNF-α (Figures S2A and B), IL-1β (Figures S2A and C), IL-6 (Figures S2A and D), and TH (Figures 3A, B and D), increased the level of GAD67 (Figures S3B and C) in the PVN in SHR + AAV EGFP NC group. These changes were not present in the SHR + Nrf2 shRNA group. These findings imply that Nrf2 knockdown can completely reverse the effects of PVN endogenous H_2S on neuroinflammation and neurotransmitter.

Discussion

In this research, we mainly looked into how oxidative stress, inflammation, and hypertensive response are affected by endogenous H_2S in PVN. Here are the main conclusions: (i) By increasing endogenous H_2S content and reducing SNA in SHR, PVN administration of AAV-CBS can alleviate hypertension; (ii) Endogenous H_2S in the PVN of SHR rats activates the Nrf2 signaling pathway, increases the production of HO-1 and SOD1, reduces oxidative stress and PICs, and balances the levels of excitatory and inhibitory neurotransmitters; (iii) PVN Nrf2 knockdown completely eliminated the ameliorative effects of endogenous H_2S on hypertension in SHR.

The gas signaling molecule hydrogen sulfide is widely distributed in the cardiovascular system, nervous system, etc. Its functions include inhibiting inflammatory response, antioxidant stress, and regulating blood pressure (Liang et al., 2017). There is much evidence that in numerous hypertension models H_2S donors and precursors can lower blood pressure (Ahmad et al., 2012; Lu et al., 2010), and reduced peripheral H_2S synthesis promotes hypertension development (Yang et al., 2008). CBS is an enzyme that produces endogenous H_2S in the CNS, which includes the PVN, cerebellum, brainstem, hippocampus, cerebral cortex, and RVLM (Kim et al., 2011; Warenycia et al., 1989). The PVN is an important nucleus that

maintains homeostasis by regulating cardiovascular and neuroendocrine functions as well as plays a key role in the management of hypertension. However, it's unclear how endogenous H_2S affects hypertension in the CNS, particularly in the PVN. According to the results of this work, CBS is expressed in the PVN, and microinjecting AAV-CBS into the PVN can boost the expression of endogenous H_2S and lower SHR blood pressure. Previous studies by our group also showed that PVN microinjection of GYY4137 (H_2S sustained-release donor) resulted in a hypotensive response in high salt-induced hypertensive rats, whereas PVN microinjection of HA (CBS inhibitor) showed an increased BP response (Liang et al., 2017). These results imply that the concentration of H_2S in PVN is related to BP and HR, and the relationship between them is negative. However, our results contradict or differ from the evidence in two other acute experiments (Gan et al., 2012; Streeter et al., 2011). One study found no significant differences in BP, HR, or lumbar SNA following bilateral microinjections into RVLM or PVN of NaHS (0.2–2000 pmol/side), CBS inhibitor amino-oxyacetate, or hydroxylamine (HA; 0.2-2.0 nmol/side) of WKY rats. The other study demonstrated that CBS activity and H_2S levels were decreased in the PVN in rats with chronic heart failure, and PVN microinjection of low doses of GYY4137 (0.01 and 0.1 nmol) had no significant effect on MAP, renal SNA, and cardiac sympathetic afferent reflex, while high doses of GYY4137 (1, 2, and 4 nmol) increased baseline renal SNA. This may be related to the fact that they were both acute experiments and ours were chronic. This may be related to the synthesis of different proteins or other substances in chronic experiments. Notably, there are also some reports supporting the hypotensive response of H_2S in the brain. Duan et al. found that chronic ICV infusion of NaHS relieved Ang II-induced hypertension, enhanced autonomic function, and had an impact on PVN microglia (Duan et al., 2015). Guo et al. found a dose-dependent decrease in MAP, renal SNA, and HR after microinjection of NaHS (4, 8, and 16 mM, 50 nl) in the bilateral RVLM (Guo et al., 2011).

Antioxidant enzyme activity and content are significantly reduced in hypertension individuals. Increased oxidative stress and blood pressure are caused by an increase in ROS levels, and hypertension further encourages an increase in ROS production and tissue oxidative damage. One of the key causes of the formation of ROS in tissues is the activation of NAD(P)H oxidase. Our earlier research has demonstrated that the development of hypertension and PVN sympathetic excitation are both significantly influenced by oxidative stress. H_2S has a powerful antioxidant function, it can directly scavenge ROS and suppress the activity of enzymes that produce ROS. H_2S inhibits ROS-mediated CHOP apoptotic signaling in endoplasmic reticulum stress (Zhu et al., 2022). Administration of GYY4137 or NaHS improved overall antioxidant capacity and raised Nrf2 levels, which together dramatically reduced immobilization-induced oxidative stress (Xu et al., 2022). Additionally, the level of phosphorylated $p47^{phox}$ was significantly reduced after NaHS infusion into RVLM, which would reduce the enzymatic activity of NADPH oxidase. Our earlier research also demonstrated that GYY4137 injection into PVN might lower the expression of NOX2, NOX4, and ROS levels (Liang et al., 2017). In this investigation, we discovered that endogenous hydrogen sulfide in the PVN decreased ROS level, expression of $gp91^{phox}$ and $p47^{phox}$, and increased expression of SOD1. This suggests that elevated H_2S levels in PVN reduce blood pressure, presumably as a result of NOX and ROS being downregulated in PVN.

At physiological concentrations, H_2S has anti-inflammatory properties that can inhibit the adhesion of leukocytes to the vascular endothelium and the migration of leukocytes to the site of inflammation. H_2S can also cause neutrophil apoptosis, drive macrophages to change to an anti-inflammatory phenotype, and decrease the production of PICs, chemokines, and enzymes. In an endotoxic shock model, H_2S can block the release of TNF-α and IL-1β. Injections of NaHS or GYY4137, according to Xu et al., reduced the infiltration of $CD45^+$ leukocytes brought on by immobilization while reducing the expression of pro-inflammatory biomarkers in skeletal muscle (Xu et al., 2022). Another study demonstrated that H_2S inhibits the activation of the NLRP3 inflammasome, which slows the development of diabetes-accelerated atherosclerosis and protects endothelial cells (Zheng et al., 2019). We investigated whether H_2S significantly affects the PICs expression in the PVN. Given bilateral injections of AAV-CBS in the PVN, we discovered that the expression levels of TNF-α, IL-6, and IL-1β were decreased while the expression levels of IL-10 were increased in SHR. Additionally, the SHR + AAV-CBS group's plasma NE levels were considerably lower, suggesting that PVN endogenous H_2S may lower blood pressure by lowering sympathetic activation. Additionally, the balance of excitatory and inhibitory neurotransmitters is off in hypertensive rats. Both glutamate and NE, the major excitatory neurotransmitters in the PVN, have been reported to increase sympathetic nerve activity, while GABA, a major inhibitory neurotransmitter, downregulates sympathetic nerve activity. Our findings are in line with earlier research showing that TH expression is upregulated whereas glutamate decarboxylase isoform GAD67, a hallmark of GABAergic neurons, is downregulated in the PVN of SHR. Endogenous H_2S in the PVN increased the GAD67 expression and decreased the TH expression. These findings imply that endogenous H_2S's ability to lower blood pressure may be due to its ability to restore the balance of neurotransmitters.

An essential antioxidant transcription factor called Nrf2 can bind to antioxidant response elements and trigger the development of antioxidant enzymes like HO-1 and SOD, which enhances the body's capacity to scavenge ROS. HO-1 is an important Nrf2 target gene. HO-1 can accelerate the degradation of heme and generate biliverdin, CO, and Fe^{2+}. Biliverdin is an endogenous antioxidant and has strong antioxidant activity (Loboda et al., 2016). According to studies, the Nrf2/HO-1 pathway negatively regulates the expression of TNF-α, IL-6, IL-1β, and cleaved caspase-3, to reduce the harm to the body caused by oxidative stress, inflammation, and apoptosis (Park et al., 2020). Nrf2 is extensively expressed in the CNS. Our research team has previously shown that activating the Nrf2 signaling pathway can shield PVN against oxidative stress by enhancing mitochondrial function (Sun et al., 2017). According to the results of the current study, endogenous H_2S in the PVN activated the Nrf2 signaling pathway in SHR, reduced levels of oxidative stress, induced antioxidant enzyme expression, and restored the balance of anti- and pro-inflammatory cytokines. Furthermore, Nrf2-shRNA in the PVN abrogated the hypotensive effect of endogenous H_2S.

Taken together, our findings imply that PVN endogenous H_2S protects against hypertension of SHR, which can be explained in part by activating the Nrf2/HO-1 pathway reducing ROS generation and PICs expression while restoring the appropriate balance of neurotransmitters in the PVN.

Declarations

Acknowledgments

This study was supported by the National Natural Science Foundation of China (Nos. 82070439, 82070440), Natural Science Research Program of Shaanxi Province (No. 2020GCZX-13) and Fundamental Research Funds for the Central University (xzy012022045).

Conflict of interest

The authors have no competing interests to declare that are relevant to the content of this article.

Author Contributions

Conceptualization: Yu-Ming Kang, Xiao-Jing Yu, Methodology: Nianping Zhang, Tingting Meng, Experiments conducted and data gathered: Wen-Jie Xia, Xiao-Min Wang, Yu Yang, Data examined and interpreted: Wen-Jie Xia, Kai-Li Liu, Jin-An Qiao, Writing - original draft preparation: Wen-Jie Xia, Xiao-Jing Yu, Writing - review and editing: Yu-Ming Kang. The finished manuscript was examined by each author.

References

1. Abd Allah, E. S. H., Ahmed, M. A., Makboul, R., & Abd El-Rahman, M. A. (2020, Apr). Effects of hydrogen sulphide on oxidative stress, inflammatory cytokines, and vascular remodelling in l-NAME-induced hypertension. *Clin Exp Pharmacol Physiol, 47*(4), 650-659. https://doi.org/10.1111/1440-1681.13240
2. Abdel-Latif, R., Heeba, G. H., Hassanin, S. O., Waz, S., & Amin, A. (2022). TLRs-JNK/ NF-kappaB Pathway Underlies the Protective Effect of the Sulfide Salt Against Liver Toxicity. *Front Pharmacol, 13*, 850066. https://doi.org/10.3389/fphar.2022.850066
3. Ahmad, F. U., Sattar, M. A., Rathore, H. A., Abdullah, M. H., Tan, S., Abdullah, N. A., & Johns, E. J. (2012). Exogenous hydrogen sulfide (H2S) reduces blood pressure and prevents the progression of diabetic nephropathy in spontaneously hypertensive rats. *Ren Fail, 34*(2), 203-210. https://doi.org/10.3109/0886022X.2011.643365
4. Bai, J., Yu, X. J., Liu, K. L., Wang, F. F., Jing, G. X., Li, H. B., Zhang, Y., Huo, C. J., Li, X., Gao, H. L., Qi, J., & Kang, Y. M. (2017, Oct 15). Central administration of tert-butylhydroquinone attenuates hypertension via regulating Nrf2 signaling in the hypothalamic paraventricular nucleus of hypertensive rats. *Toxicol Appl Pharmacol, 333*, 100-109. https://doi.org/10.1016/j.taap.2017.08.012
5. Donertas Ayaz, B., Oliveira, A. C., Malphurs, W. L., Redler, T., de Araujo, A. M., Sharma, R. K., Sirmagul, B., & Zubcevic, J. (2021). Central Administration of Hydrogen Sulfide Donor NaHS Reduces Iba1-Positive Cells in the PVN and Attenuates Rodent Angiotensin II Hypertension. *Front Neurosci, 15*, 690919. https://doi.org/10.3389/fnins.2021.690919

6. Duan, X. C., Guo, R., Liu, S. Y., Xiao, L., Xue, H. M., Guo, Q., Jin, S., & Wu, Y. M. (2015, Mar 15). Gene transfer of cystathionine beta-synthase into RVLM increases hydrogen sulfide-mediated suppression of sympathetic outflow via KATP channel in normotensive rats. *Am J Physiol Heart Circ Physiol, 308*(6), H603-611. https://doi.org/10.1152/ajpheart.00693.2014
7. Gan, X. B., Liu, T. Y., Xiong, X. Q., Chen, W. W., Zhou, Y. B., & Zhu, G. Q. (2012). Hydrogen sulfide in paraventricular nucleus enhances sympathetic activity and cardiac sympathetic afferent reflex in chronic heart failure rats. *PLoS One, 7*(11), e50102. https://doi.org/10.1371/journal.pone.0050102
8. Gao, H. L., Yu, X. J., Hu, H. B., Yang, Q. W., Liu, K. L., Chen, Y. M., Zhang, Y., Zhang, D. D., Tian, H., Zhu, G. Q., Qi, J., & Kang, Y. M. (2021, Sep). Apigenin Improves Hypertension and Cardiac Hypertrophy Through Modulating NADPH Oxidase-Dependent ROS Generation and Cytokines in Hypothalamic Paraventricular Nucleus. *Cardiovasc Toxicol, 21*(9), 721-736. https://doi.org/10.1007/s12012-021-09662-1
9. Gao, L., Zimmerman, M. C., Biswal, S., & Zucker, I. H. (2017, Jun). Selective Nrf2 Gene Deletion in the Rostral Ventrolateral Medulla Evokes Hypertension and Sympathoexcitation in Mice. *Hypertension, 69*(6), 1198-1206. https://doi.org/10.1161/HYPERTENSIONAHA.117.09123
10. Guo, Q., Jin, S., Wang, X. L., Wang, R., Xiao, L., He, R. R., & Wu, Y. M. (2011, Aug). Hydrogen sulfide in the rostral ventrolateral medulla inhibits sympathetic vasomotor tone through ATP-sensitive K+ channels. *J Pharmacol Exp Ther, 338*(2), 458-465. https://doi.org/10.1124/jpet.111.180711
11. Hsu, C. N., Hou, C. Y., Chang-Chien, G. P., Lin, S., Yang, H. W., & Tain, Y. L. (2022, Jan 11). Sodium Thiosulfate Improves Hypertension in Rats with Adenine-Induced Chronic Kidney Disease. *Antioxidants (Basel), 11*(1). https://doi.org/10.3390/antiox11010147
12. Hsu, C. N., Lin, Y. J., Lu, P. C., & Tain, Y. L. (2018, Jan). Early Supplementation of d-Cysteine or l-Cysteine Prevents Hypertension and Kidney Damage in Spontaneously Hypertensive Rats Exposed to High-Salt Intake. *Mol Nutr Food Res, 62*(2). https://doi.org/10.1002/mnfr.201700596
13. Javkhedkar, A. A., Quiroz, Y., Rodriguez-Iturbe, B., Vaziri, N. D., Lokhandwala, M. F., & Banday, A. A. (2015, May 15). Resveratrol restored Nrf2 function, reduced renal inflammation, and mitigated hypertension in spontaneously hypertensive rats. *Am J Physiol Regul Integr Comp Physiol, 308*(10), R840-846. https://doi.org/10.1152/ajpregu.00308.2014
14. Kang, Y., Ding, L., Dai, H., Wang, F., Zhou, H., Gao, Q., Xiong, X., Zhang, F., Song, T., Yuan, Y., Zhu, G., & Zhou, Y. (2019, Aug 28). Intermedin in Paraventricular Nucleus Attenuates Ang II-Induced Sympathoexcitation through the Inhibition of NADPH Oxidase-Dependent ROS Generation in Obese Rats with Hypertension. *Int J Mol Sci, 20*(17). https://doi.org/10.3390/ijms20174217
15. Kim, B., Lee, J., Jang, J., Han, D., & Kim, K. H. (2011, May 5). Prediction on the seasonal behavior of hydrogen sulfide using a neural network model. *ScientificWorldJournal, 11*, 992-1004. https://doi.org/10.1100/tsw.2011.95
16. Li, M., Hu, W., Wang, R., Li, Z., Yu, Y., Zhuo, Y., Zhang, Y., Wang, Z., Qiu, Y., Chen, K., Ding, Q., Qi, W., Zhu, M., & Zhu, Y. (2022, Apr 7). Sp1 S-Sulfhydration Induced by Hydrogen Sulfide Inhibits Inflammation

via HDAC6/MyD88/NF-kappaB Signaling Pathway in Adjuvant-Induced Arthritis. *Antioxidants (Basel), 11*(4). https://doi.org/10.3390/antiox11040732

17. Liang, Y. F., Zhang, D. D., Yu, X. J., Gao, H. L., Liu, K. L., Qi, J., Li, H. B., Yi, Q. Y., Chen, W. S., Cui, W., Zhu, G. Q., & Kang, Y. M. (2017, Mar 15). Hydrogen sulfide in paraventricular nucleus attenuates blood pressure by regulating oxidative stress and inflammatory cytokines in high salt-induced hypertension. *Toxicol Lett, 270*, 62-71. https://doi.org/10.1016/j.toxlet.2017.02.004
18. Liu, Z., Wang, X., Li, L., Wei, G., & Zhao, M. (2020). Hydrogen Sulfide Protects against Paraquat-Induced Acute Liver Injury in Rats by Regulating Oxidative Stress, Mitochondrial Function, and Inflammation. *Oxid Med Cell Longev, 2020*, 6325378. https://doi.org/10.1155/2020/6325378
19. Loboda, A., Damulewicz, M., Pyza, E., Jozkowicz, A., & Dulak, J. (2016, Sep). Role of Nrf2/HO-1 system in development, oxidative stress response and diseases: an evolutionarily conserved mechanism. *Cell Mol Life Sci, 73*(17), 3221-3247. https://doi.org/10.1007/s00018-016-2223-0
20. Lu, M., Liu, Y. H., Goh, H. S., Wang, J. J., Yong, Q. C., Wang, R., & Bian, J. S. (2010, Jun). Hydrogen sulfide inhibits plasma renin activity. *J Am Soc Nephrol, 21*(6), 993-1002. https://doi.org/10.1681/ASN.2009090949
21. Park, C., Lee, H., Noh, J. S., Jin, C. Y., Kim, G. Y., Hyun, J. W., Leem, S. H., & Choi, Y. H. (2020, Sep 30). Hemistepsin A protects human keratinocytes against hydrogen peroxide-induced oxidative stress through activation of the Nrf2/HO-1 signaling pathway. *Arch Biochem Biophys, 691*, 108512. https://doi.org/10.1016/j.abb.2020.108512
22. Rostami, B., & Hatam, M. (2022, May). Central Nucleus of Amygdala Mediate Pressor Response Elicited by Microinjection of Angiotensin II into the Parvocellular Paraventricular Nucleus in Rats. *Iran J Med Sci, 47*(3), 272-279. https://doi.org/10.30476/ijms.2021.90015.2080
23. Sikora, M., Drapala, A., & Ufnal, M. (2014, Oct). Exogenous hydrogen sulfide causes different hemodynamic effects in normotensive and hypertensive rats via neurogenic mechanisms. *Pharmacol Rep, 66*(5), 751-758. https://doi.org/10.1016/j.pharep.2014.04.004
24. Streeter, E., Al-Magableh, M., Hart, J. L., & Badoer, E. (2011). Hydrogen Sulfide in the RVLM and PVN has No Effect on Cardiovascular Regulation. *Front Physiol, 2*, 55. https://doi.org/10.3389/fphys.2011.00055
25. Su, Q., Liu, J. J., Cui, W., Shi, X. L., Guo, J., Li, H. B., Huo, C. J., Miao, Y. W., Zhang, M., Yang, Q., & Kang, Y. M. (2016, Jan 22). Alpha lipoic acid supplementation attenuates reactive oxygen species in hypothalamic paraventricular nucleus and sympathoexcitation in high salt-induced hypertension. *Toxicol Lett, 241*, 152-158. https://doi.org/10.1016/j.toxlet.2015.10.019
26. Su, Q., Yu, X. J., Wang, X. M., Li, H. B., Li, Y., Bai, J., Qi, J., Zhang, N., Liu, K. L., Zhang, Y., Zhu, G. Q., & Kang, Y. M. (2021). Bilateral Paraventricular Nucleus Upregulation of Extracellular Superoxide Dismutase Decreases Blood Pressure by Regulation of the NLRP3 and Neurotransmitters in Salt-Induced Hypertensive Rats. *Front Pharmacol, 12*, 756671. https://doi.org/10.3389/fphar.2021.756671

27. Sun, W., Wang, X., Hou, C., Yang, L., Li, H., Guo, J., Huo, C., Wang, M., Miao, Y., Liu, J., & Kang, Y. (2017, Feb). Oleuropein improves mitochondrial function to attenuate oxidative stress by activating the Nrf2 pathway in the hypothalamic paraventricular nucleus of spontaneously hypertensive rats. *Neuropharmacology, 113*(Pt A), 556-566. https://doi.org/10.1016/j.neuropharm.2016.11.010
28. Tapia, E., Soto, V., Ortiz-Vega, K. M., Zarco-Marquez, G., Molina-Jijon, E., Cristobal-Garcia, M., Santamaria, J., Garcia-Nino, W. R., Correa, F., Zazueta, C., & Pedraza-Chaverri, J. (2012). Curcumin induces Nrf2 nuclear translocation and prevents glomerular hypertension, hyperfiltration, oxidant stress, and the decrease in antioxidant enzymes in 5/6 nephrectomized rats. *Oxid Med Cell Longev, 2012*, 269039. https://doi.org/10.1155/2012/269039
29. Warenycia, M. W., Goodwin, L. R., Benishin, C. G., Reiffenstein, R. J., Francom, D. M., Taylor, J. D., & Dieken, F. P. (1989, Mar 15). Acute hydrogen sulfide poisoning. Demonstration of selective uptake of sulfide by the brainstem by measurement of brain sulfide levels. *Biochem Pharmacol, 38*(6), 973-981. https://doi.org/10.1016/0006-2952(89)90288-8
30. Wu, K. L. H., Wu, C. W., Chao, Y. M., Hung, C. Y., & Chan, J. Y. H. (2016, Aug). Impaired Nrf2 regulation of mitochondrial biogenesis in rostral ventrolateral medulla on hypertension induced by systemic inflammation. *Free Radic Biol Med, 97*, 58-74. https://doi.org/10.1016/j.freeradbiomed.2016.05.012
31. Xia, W. J., Xu, M. L., Yu, X. J., Du, M. M., Li, X. H., Yang, T., Li, L., Li, Y., Kang, K. B., Su, Q., Xu, J. X., Shi, X. L., Wang, X. M., Li, H. B., & Kang, Y. M. (2021, Jan-Dec). Antihypertensive effects of exercise involve reshaping of gut microbiota and improvement of gut-brain axis in spontaneously hypertensive rat. *Gut Microbes, 13*(1), 1-24. https://doi.org/10.1080/19490976.2020.1854642
32. Xu, M., Liu, X., Bao, P., Wang, Y. J., Lu, J., & Liu, Y. J. (2022). H2S Protects Against Immobilization-Induced Muscle Atrophy via Reducing Oxidative Stress and Inflammation. *Front Physiol, 13*, 844539. https://doi.org/10.3389/fphys.2022.844539
33. Yang, G., Wu, L., Jiang, B., Yang, W., Qi, J., Cao, K., Meng, Q., Mustafa, A. K., Mu, W., Zhang, S., Snyder, S. H., & Wang, R. (2008, Oct 24). H2S as a physiologic vasorelaxant: hypertension in mice with deletion of cystathionine gamma-lyase. *Science, 322*(5901), 587-590. https://doi.org/10.1126/science.1162667
34. Yu, H., Xu, H., Liu, X., Zhang, N., He, A., Yu, J., & Lu, N. (2015). Superoxide Mediates Depressive Effects Induced by Hydrogen Sulfide in Rostral Ventrolateral Medulla of Spontaneously Hypertensive Rats. *Oxid Med Cell Longev, 2015*, 927686. https://doi.org/10.1155/2015/927686
35. Yu, X. J., Liu, X. J., Guo, J., Su, Y. K., Zhang, N., Qi, J., Li, Y., Fu, L. Y., Liu, K. L., Li, Y., & Kang, Y. M. (2022, Apr 19). Blockade of Microglial Activation in Hypothalamic Paraventricular Nucleus Improves High-salt Induced Hypertension. *Am J Hypertens*. https://doi.org/10.1093/ajh/hpac052
36. Yu, X. J., Xin, G. R., Liu, K. L., Liu, X. J., Fu, L. Y., Qi, J., Kang, K. B., Meng, T. T., Yi, Q. Y., Li, Y., Sun, Y. J., & Kang, Y. M. (2021). Paraventricular Nucleus Infusion of Oligomeric Proantho Cyanidins Improves Renovascular Hypertension. *Front Neurosci, 15*, 642015. https://doi.org/10.3389/fnins.2021.642015
37. Zheng, Q., Pan, L., & Ji, Y. (2019, Aug 30). H 2S protects against diabetes-accelerated atherosclerosis by preventing the activation of NLRP3 inflammasome. *J Biomed Res, 34*(2), 94-102.

https://doi.org/10.7555/JBR.33.20190071

38. Zhu, S., Li, X., Dang, B., Wu, F., Gou, K., Wang, C., & Lin, C. (2022, Dec). Hydrogen sulfide protects retina from blue light-induced photodamage and degeneration via inhibiting ROS-mediated ER stress-CHOP apoptosis signal. *Redox Rep, 27*(1), 100-110. https://doi.org/10.1080/13510002.2022.2069534

Figures

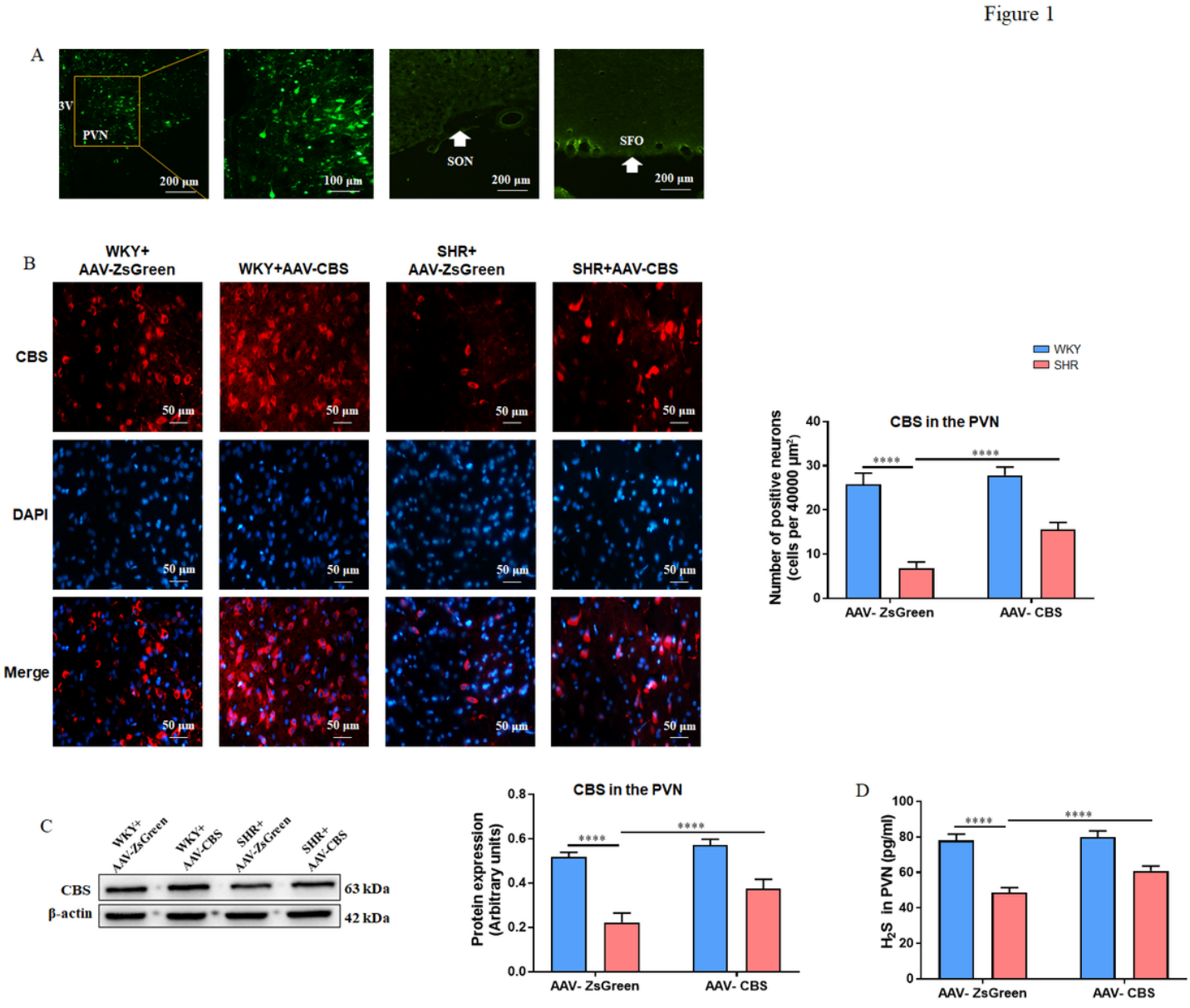

Figure 1

Effects of AAV-CBS in the PVN on CBS expression and H_2S level blood pressure. (A) Green fluorescent protein ZsGreen fluorescence images of the PVN, supraoptic nucleus (SON), and subfornical organ as examples (SFO). (B) Representative immunofluorescence images at 40x magnification showing double

staining of CBS (red) and nucleus (blue) in the PVN, and the summary data for the CBS-positive neurons in four groups. (C) The representative immunoblot images for CBS in the PVN, and quantification of western blotting images for CBS in four groups. (D) Measurement of H_2S level in the PVN in four groups. 3 V, the third ventricle.n = 6-8/group. Values are mean ± SEM. **P*<0.05, ***P*<0.01, ****P*<0.001, *****P*<0.0001.

Figure 2

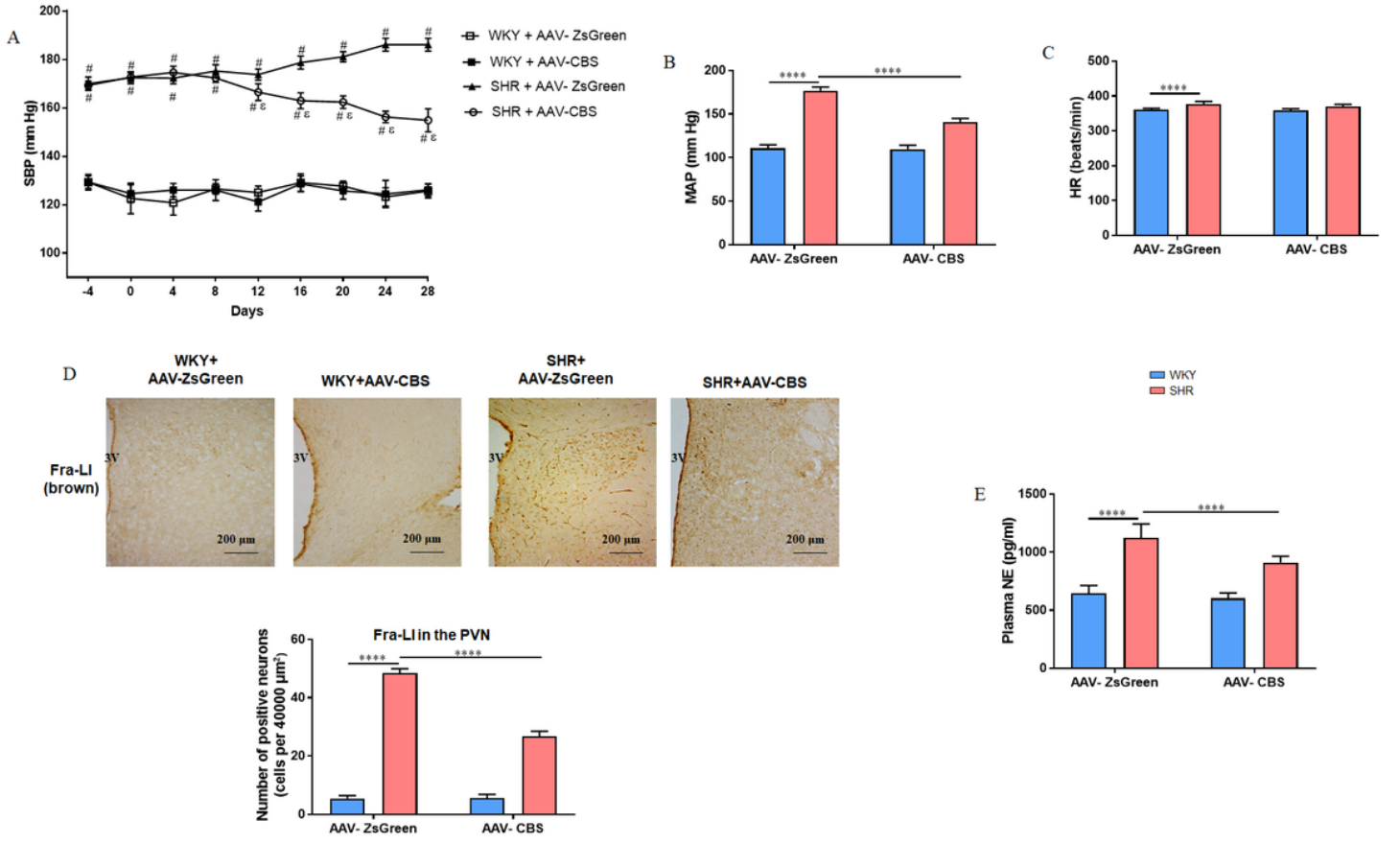

Figure 2

Effects of AAV-CBS in the PVN on blood pressure, heart rate, PVN Fra-LI expression and plasma NE. (A) Time course of systolic blood pressure (SBP)in four groups. (B) Mean arterial blood pressure (MAP) was assessed at the end of the experiment. (C) Measurement of heart rate in four groups. (D) Representative immunohistochemistry images at 10x magnification showing staining of Fra-LI (brown) in the PVN, and the summary data for Fra-LI in the PVN in four groups. (E) Measurement of NE level in plasma in four groups. 3 V, the third ventricle.n = 6-8/group. Values are mean ± SEM. In SBP data: #*P*< 0.05 versus WKY groups (WKY + AAV- ZsGreen or WKY + AAV-CBS), ε*P*<0.05 versus SHR + AAV- ZsGreen. In other parameters: **P*<0.05, ***P*<0.01, ****P*<0.001, *****P*<0.0001.

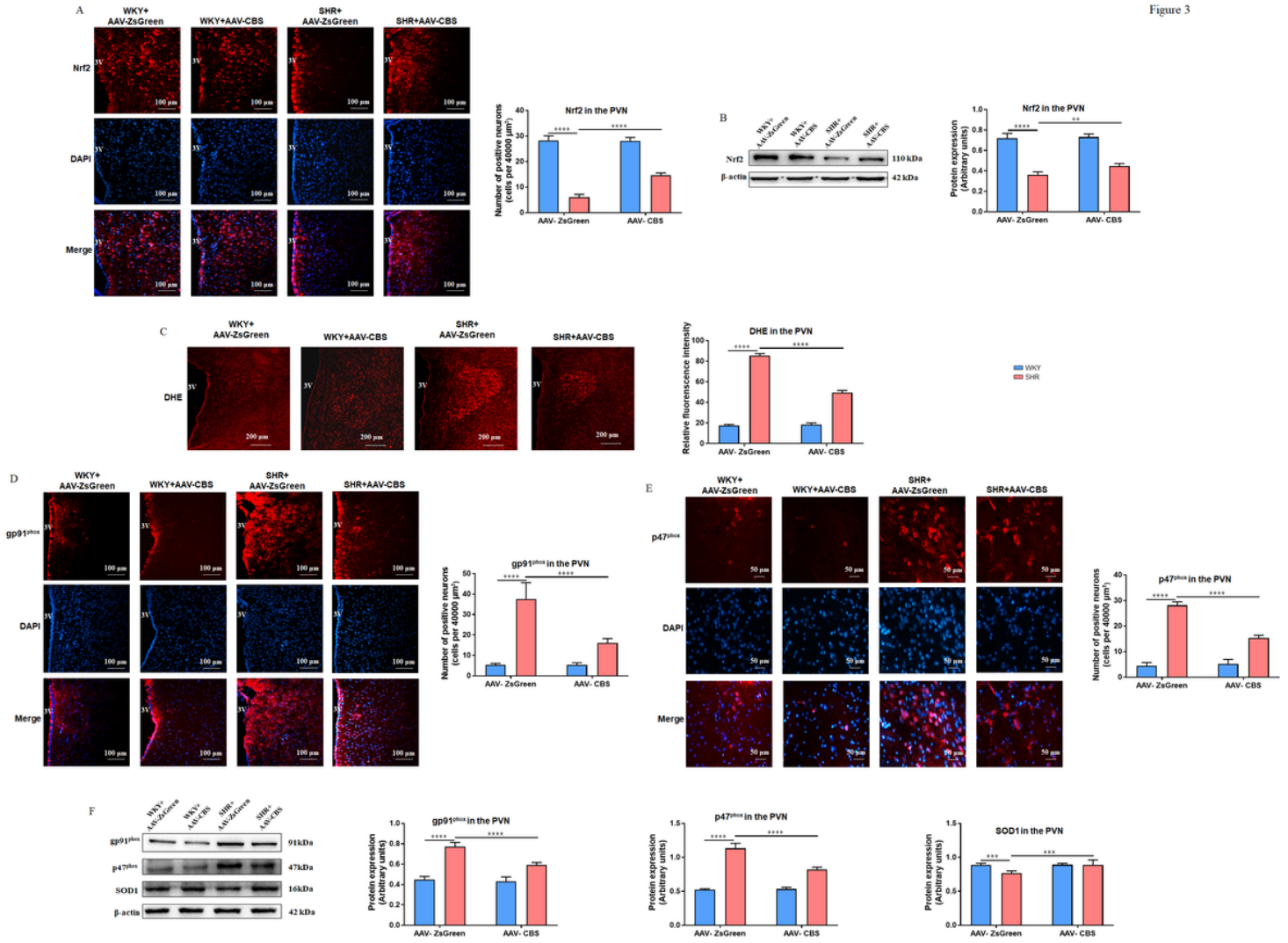

Figure 3

Effects of endogenous H_2S in the PVN on Nrf2 expression and oxidative stress. (A) Representative immunofluorescence images at 20x magnification showing double staining of Nrf2 (red) and nucleus (blue) in the PVN, and the summary data for the Nrf2 in the PVN in four groups. (B) The representative immunoblot images for Nrf2, and quantification of western blotting images for Nrf2 in the PVN in four groups. (C) An illustration of DHE staining in the PVN at 10x magnification, and the data of DHE staining in different groups. (D) Representative immunofluorescence images at 20x magnification showing double staining of gp91^{phox}-positive neurons (red) and nucleus (blue) in the PVN, and the summary data for the gp91^{phox} in the PVN in four groups. (E) Representative immunofluorescence images at 20x magnification showing double staining of p47^{phox}-positive neurons (red) and nucleus (blue) in the PVN, and the summary data for the p47^{phox} in the PVN in four groups. (F) The representative immunoblot images for gp91^{phox}, p47^{phox,} and SOD1, and quantification of western blotting images for gp91^{phox}, p47^{phox}, and SOD1 in the PVN in four groups. 3 V, the third ventricle. n = 6-8/group. Values are mean ± SEM. *P<0.05, **P<0.01, ***P<0.001, ****P<0.0001.

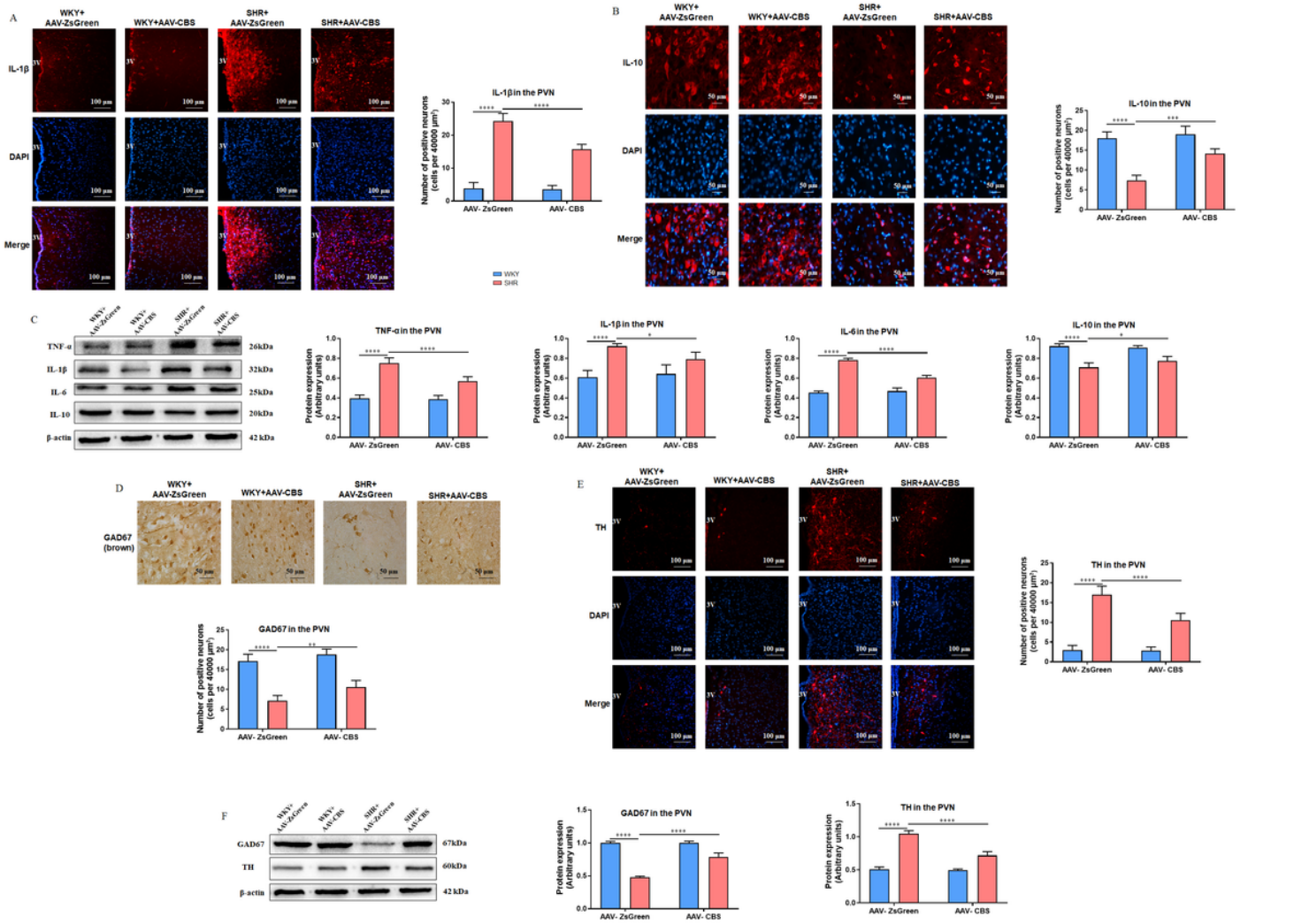

Figure 4

Effects of endogenous H_2S in the PVN on inflammation and neurotransmitter. (A) Representative immunofluorescence images at 20x magnification showing double staining of IL-1β (red) and nucleus (blue) in the PVN, and the summary data for the IL-1β in the PVN in four groups. (B) Representative immunofluorescence images at 40x magnification showing double staining of IL-10 (red) and nucleus (blue) in the PVN, and the summary data for the IL-10 in the PVN in four groups. (C) The representative immunoblot images for TNF-α, IL-1β, IL-6, and IL-10, and quantification of western blotting images for TNF-α, IL-1β, IL-6, and IL-10 in the PVN in different groups. (D) Representative immunohistochemistry images at 40x magnification showing staining of GAD67 (brown) in the PVN, and the summary data for the GAD67 in the PVN in four groups. (E) Representative immunofluorescence images at 20x magnification showing double staining of TH (red) and nucleus (blue) in the PVN, and the summary data for the TH in the PVN in four groups. (F) The representative immunoblots images for GAD67 and TH, and quantification of western blotting images for GAD67, and TH in the PVN in different groups. 3 V, the third ventricle. n = 6-8/group. Values are mean ± SEM. **P*<0.05, ***P*<0.01, ****P*<0.001, *****P*<0.0001.

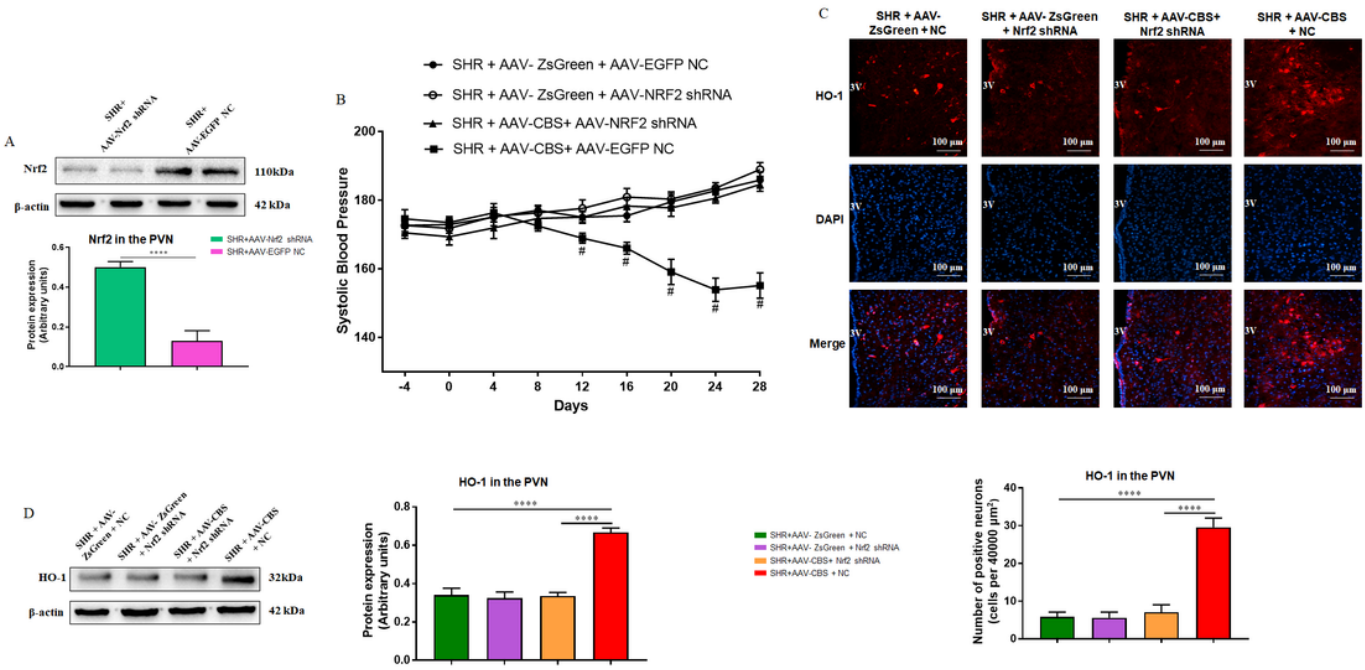

Figure 5

Effects of AAV-shNrf2 in the PVN on Nrf2 expression, blood pressure, and HO-1 expression. (A) The representative immunoblot image for Nrf2, and quantification of western blotting images for Nrf2 in the PVN in four groups. (B) Time course of SBP in four groups. (C) Representative immunofluorescence images at 20x magnification showing double staining of HO-1 (red) and nucleus (blue) in the PVN, and the summary data for the HO-1 in the PVN in four groups. (D) The representative immunoblot images for HO-1, and quantification of western blotting images for HO-1 in the PVN in different groups. 3 V, the third ventricle. n = 6-8/group. Values are mean ± SEM. In SBP data: #P<0.05 versus SHR +AAV-CBS + AAV-Nrf2 shRNA. In other parameters: *P<0.05, **P<0.01, ***P<0.001, ****P<0.0001.

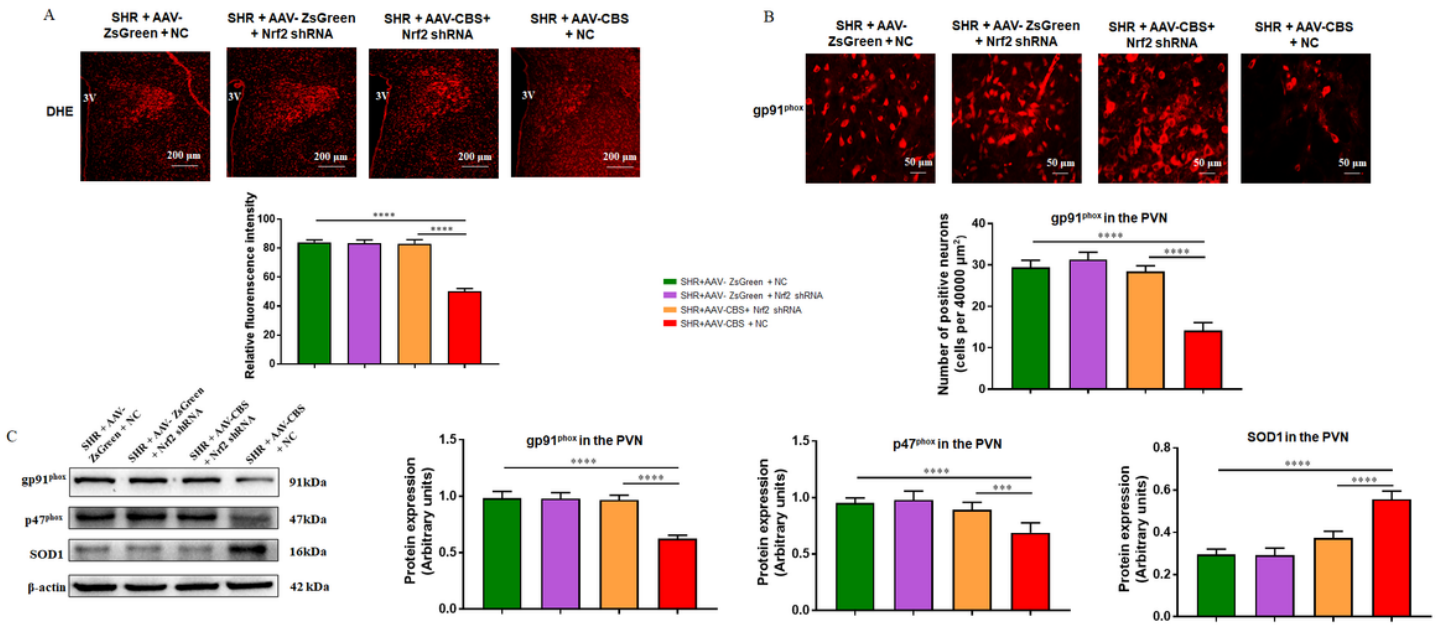

Figure 6

Effects of PVN knockdown Nrf2 on oxidative stress. (A) An illustration of DHE staining in the PVN at 10x magnification, and the data of DHE staining in different groups. (B) Representative immunofluorescence images at 40x magnification showing staining of $gp91^{phox}$ (red) in the PVN, and the summary data for the $gp91^{phox}$ in the PVN in four groups. (C) The representative immunoblot images for $gp91^{phox}$, $p47^{phox}$, and SOD1, and quantification of western blotting images for $gp91^{phox}$, $p47^{phox}$, and SOD1 in the PVN in four groups. 3 V, the third ventricle. n = 6-8/group. Values are mean ± SEM. **P*<0.05, ***P*<0.01, ****P*<0.001, *****P*<0.0001.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- SupplementaryMaterial.doc
- figures1.tif
- figures2.tif
- figures3.tif