Overexpressed C1q-TNF-related protein-9 in hypothalamic paraventricular nucleus ameliorates hypertension through modulating cytokines and attenuating oxidative stress in spontaneously hypertensive rats

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Research Article

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Abstract

The adipocytokine C1q/TNF-related protein 9 (CTRP9) was recently reported to have multiple protective effects on cardiac and cardiovascular function. However, the exact role of CTRP9 in the progression of hypertension remains unclear. This study was designed to determine whether overexpressed CTRP9 in hypothalamic paraventricular nucleus (PVN) attenuates hypertension through modulating pro-inflammatory

cytokines and attenuating oxidative stress in spontaneously hypertensive rats (SHRs). Our data on enzyme-linked immunosorbent assay and real-time quantitative polymerase chain reaction showed that CTRP9 mRNA and protein content were markedly downregulated in SHRs. SHRs had higher mean arterial pressure (MAP) and plasma norepinephrine (NE). These rats also had higher levels of tumor necrosis factor alpha (TNF)-α, interleukin-1beta (IL-1β), interleukin-6 (IL-6), monocyte chemotactic protein-1 (MCP-1), and higher gp^{91phox}, gp^{47phox} (subunits of NAD (P)H oxidase) expression and superoxide production in PVN. Bilateral PVN microinjection of adeno-associated virus (AAV)-CTRP9 significantly attenuated MAP and NE, also reduced the expressions of TNF-α, IL-1β, IL-6, MCP-1, gp^{91phox} and gp^{47phox} in PVN of SHRs, but not in WKY rats. The mRNA expression of TNF-α, IL-1β, IL-6, MCP-1, gp^{91phox} and gp^{47phox} are in accordance with their protein expression. In conclusion, CTRP9 in PVN ameliorated hypertension may through modulating cytokines and attenuating oxidative stress in spontaneously hypertensive rats.

Introduction

Hypertension is one of most common chronic diseases and one of the most important risk factors for cardiovascular and cerebrovascular diseases. The hypothalamic paraventricular nucleus (PVN) has been well known as the arterial blood pressure control center, and a predominant region for coordinating nervous signals regulating blood pressure[1, 2]. The PVN also plays a crucial role in the development of hypertension. A growing body of evidence indicates that various pro-inflammatory cytokines (PICs) such as tumor necrosis factor alpha (TNF)-α, interleukin-1beta (IL-1β), interleukin-6 (IL-6) and monocyte chemotactic protein-1 (MCP-1) within the PVN of the brain play an important role in the development of hypertension[1, 3, 4]. These studies suggest that PICs in the PVN are closely related to the regulation of blood pressure and renal sympathetic nerve activity in the progress of hypertension. Recent evidence also suggests that reactive oxygen species (ROS) plays a critical role in the regulation of sympathoexcitation and hypertensive response in the PVN[5–7]. ROS are increased in the PVN of hypertensive rats and that blockade of ROS decreases sympathetic activity. NADPH oxidase is a major source of ROS in hypertension and plays a critical role in generating ROS in the brain[8–11]. Recent studies have found high salt diet produces excessive amount of ROS and increases the expression of superoxide protein gp^{91phox} and gp^{47phox} (amembrane associated oxidase protein). Blocking the production of ROS in the PVN will contribute to the prevention of hypertension [7, 12, 13]. However, the effect of PVN microinjection of adeno-associated virus (AAV)-CTRP9 on PICs and ROS during hypertension remains unclear.

C1q/tumor necrosis factor-related protein-9 (CTRP9) is a newly discovered adipocytokine belonging to the CTRP protein family and has a similar structure to adiponectin[14]. CTRP9 is involved in lipid metabolism and cardiovascular protection[15, 16]. Therefore, CTRP9 has a protective effect on the cardiovascular system, through relaxing vessel, relieving inflammatory reaction, and improving vascular epithelial cell survival[17]. Recent evidence also suggests that CTRP9 delayed the progression of hypertension-related arteriosclerosis by alleviating endothelial dysfunction[18–20]. However, the exact role of CTRP9 in the progression of hypertension remains still unclear.

Considering the effects of pro-inflammatory cytokine and reactive oxygen species in hypertension pathogenesis, we hypothesized that CTRP9 could attenuates hypertension through modulating cytokines and attenuating oxidative stress. We tested the effects of bilateral PVN microinjection of adeno-associated virus (AAV)-CTRP9 on the expression of the proinflammatory cytokine and reactive oxygen species in spontaneously hypertensive rats.

Materials And Methods

Animal

Twelve-week-old male spontaneously hypertensive rats (SHR) and Wistar-Kyoto (WKY) rats were purchased from Charles River Laboratories. The rats were housed in a climate-controlled room with a 12 h light-dark cycle and allowed access to standard rat chow and tap water ad libitum. All study protocols were approved by the Institutional Animal Care and Use Committee, Xi'an university. The design conformed to the Guide for the Care and Use of Laboratory Animals published by the United States National Institutes of Health (NIH Publication No. 85 – 23, revised 1996).

Adenovirus-associated virus preparation

Adenoassociated virus (AAV) is an efficient and safe vector for gene transfer *in vivo*[21]. Thus, the fragment comprising the entire protein-coding region of rat CTRP9 was cloned into the AAV vector (pHBAAV-CMV-MCS-3flag-EF1-ZsGreen), which was driven by a cytomegalovirus promoter and harbored a GFP region. Recombinant AAV was plaque-purified, and the titer measured by a plaque assay on cells in culture at 1×10^{12} viral genomes. After we obtained the denovirus-associated virus, the AAV would be subpackaged (200 μl/tube) and store at -80°C. Before bilateral paraventricular nucleus injection of AAV-CMV-CTRP9 or AAV-CMV-GFP, the vectors should be dissolved on ice. All procedures were undertaken by Hanbio Biotechnology Co. Ltd (Shanghai, China,).

General experimental protocol

One week after habituation to their new environment, rats were divided randomly into four groups: (1) SHR + PVN AAV-CTRP9, (2) SHR + PVN AAV-GFP, (3) WKY + PVN AAV-CTRP9, (4) WKY + PVN AAV-GFP. Rats were anaesthetized by intraperitoneal injection with a mixture of ketamine (80 mg/kg) and xylazine (10 mg/kg), and their heads were placed in a stereotaxic instrument. Each group of rats were

administered with bilateral PVN microinjection of the AAV-CMV-CTRP9 or AAV-CMV-GFP. The microinjector (5 μl) with vectors (either AAV-CMV-CTRP9 or AAV-CMV-GFP) were installed in the microinjection pump. Vectors were bilaterally injected into the PVN (coordinates:±0.4 mm from midline, -1.8 mm posterior to bregma, and − 7.9 mm ventral to dura according to the Paxinos and Watson rat brain atlas) using 1 μl volume each side at the rate of 0.1 μl/min[22–24]. At the end of the study, after rats were anesthetized, blood was collected from the abdominal aortic vein and centrifuged at 3,000 r/min to obtain the plasma. The PVN tissue was isolated following Palkovits's microdissection procedure as previously described[25, 26]. One part of fresh brain tissues was put into 4% paraformaldehyde for 3 days and then moved into 30% sucrose for dehydration. Plasma and other brain tissues were stored at -80°C for future analysis.

Measurement of mean arterial pressure

During the 18 days experimental period, mean arterial pressure (MAP) was measured two days in the SHR and WKY rats. All rats were habituated to the blood pressure measuring system and to the holders daily for two days prior to the initiation of experimental measurements. Each rat was allowed to accommodate the cuff for 10 minutes before the blood pressure measurement. Blood pressure values were averaged from five consecutive cycles per day obtained from each rat.

Immunofluorescence

The methods for immunofluorescence and immunohistochemistry studies were performed as described previously to immunolocalize $gp91^{phox}$, $gp47^{phox}$, IL-1β and IL-6 expressions in the PVN. The primary antibodies for $gp91^{phox}$ (ab280952, a Polyclonal Rabbit Anti-Rat) and $gp47^{phox}$ (ab133303, a Polyclonal Rabbit Anti-Rat) were purchased from Abcam. The primary antibodies for IL-1β (sc-1251, a Polyclonal Goat Anti-Rat) and IL-6 (sc-1265, a Polyclonal Goat Anti-Rat) were purchased from Santa Cruz Biotechnology.

Dihydroethidium staining

Dihydroethidium (DHE) staining was used to determine the superoxide generation and the staining protocol was performed as described[27]. Briefly, free-floating sections containing PVN were incubated with DHE (0.05 mM) at 37°C for 30 min, then sections were rinsed in 0.01 M PBS for three times. Sections were imaged using a Nikon epifluorescence microscope.

Western blotting

Western blotting was used for measurement of MCP-1, TNF-α, IL-6 and IL-1β expressions in the PVN. The methods for Western blotting studies were performed as described previously[28, 29]. Protein loading was controlled by probing all blots with β-actin antibody (Thermo Scientific, USA) and normalizing their protein intensities to that of β-actin. Band densities were analyzed with NIH Image J software.

Real-time PCR.

Total RNA was extracted from microdissected PVN using TRIzol reagent (Invitrogen) and reverse transcribed using oligo (dT) with conditions at 23℃ for 10 min, 37℃ for 60 min, and 95℃ for 5 min. The cDNA used for real-time PCR with specific primers for CTRP9, TNF-α, IL-1β, IL-6, MCP-1, $gp91^{phox}$, $gp47^{phox}$ and GAPDH were shown in Table 1[30–32]. The quantitative fold changes in mRNA expression were determined relative to GAPDH mRNA levels in each corresponding group.

ELISA measurement of CTRP9 in the PVN and plasma NE

The levels of serum CTRP9 and plasma NE were quantified using commercially available rat ELISA kits (Invitrogen) according to the manufacturer's instructions.

Statistical analysis

All data were expressed as mean ± SEM. Statistical analysis was done by one-way ANOVA followed by a Tukey post-hoc test. Blood pressure data were analyzed by repeated measures ANOVA. A probability value of $P < 0.05$ was considered to be statistically significant.

Results

Effect of PVN microinjection of Ad-CTRP9 on mean arterial pressure in SHRs

The mean arterial pressure(MAP) in SHR was significantly increased compared with those in WKYs. Whereas bilateral PVN microinjection of Ad-CTRP9 attenuated MAP from day 8 when compared with PVN microinjection of Ad-GFP in SHRs, while still higher than that of the WKY groups. (Fig. 1)

Effect of PVN microinjection of Ad-CTRP9 on serum CTRP9 and mRNA levels of CTRP9 in SHRs

The serum CTRP9 and mRNA levels of CTRP9 in SHRs were much more than those in WKYs groups. Bilateral PVN microinjection of Ad-CTRP9 significantly decreased the serum CTRP9 and mRNA levels of CTRP9 in SHRs. (Fig. 2)

Effect of PVN microinjection of Ad-CTRP9 on mRNA levels of TNF-α, IL-1β, IL-6 and MCP-1 in the PVN

The mRNA levels of TNF-α, IL-1β, IL-6 and MCP-1 were increased in the PVN of SHRs compared with those of WKYs. However, bilateral PVN microinjection of Ad-CTRP9 significantly decreased the mRNA levels of TNF-α, IL-1β, IL-6 and MCP-1 in SHRs compared with those of WKYs. (Fig. 3)

Effect of PVN microinjection of Ad-CTRP9 on $gp91^{phox}$ and $gp47^{phox}$ positive neurons in the PVN

Immunofluorescence studies revealed that the number of $gp91^{phox}$and $gp47^{phox}$ positive neurons were increased in the PVN of SHRs compared with those of WKYs. However, bilateral PVN microinjection of Ad-CTRP9 significantly decreased the the number of $gp91^{phox}$and $gp47^{phox}$ positive neurons in the PVN in SHRs compared with those of WKYs. (Fig. 4)

Effect of PVN microinjection of Ad-CTRP9 on Oxidative Stress in the PVN of SHRs

The DHE staining was conducted to reflect superoxide anion production. SHR rats revealed higher levels of DHE in the PVN than those of WKY rats, whereas PVN microinjection of Ad-CTRP9 attenuated DHE in the PVN of SHR rats. (Fig. 5)

Effect of PVN microinjection of Ad-CTRP9 on IL-1β and IL-6 positive neurons in the PVN

Immunofluorescence results showed that the number of IL-1β and IL-6 positive neurons in SHRs were much more than those in WKYs groups. Bilateral PVN microinjection of Ad-CTRP9 significantly decreased the number of IL-1β and IL-6 positive neurons in the PVN in SHRs compared with those of WKYs. (Fig. 6)

Effect of PVN microinjection of Ad-CTRP9 on protein levels of MCP-1, TNF-α, IL-6 and IL-1β in the PVN

Western blotting results showed that the expressions of MCP-1, TNF-α, IL-6 and IL-1β in PVN in SHRs were much more than those in WKYs groups. However, bilateral PVN microinjection of Ad-CTRP9 decreased the expressions of MCP-1, TNF-α, IL-6 and IL-1β in SHRs compared with those of WKYs. (Fig. 7)

Effect of PVN microinjection of Ad-CTRP9 on mRNA levels of $gp91^{phox}$ and $gp47^{phox}$ in SHRs, and plasma noradrenaline (NE) in SHRs

The mRNA levels of $gp91^{phox}$ and $gp47^{phox}$ were increased in the PVN of SHRs compared with those of WKYs. However, bilateral PVN microinjection of Ad-CTRP9 significantly decreased the mRNA levels of $gp91^{phox}$ and $gp47^{phox}$ in SHRs compared with those of WKYs. The level of plasma NE was increased in SHRs compared with that of WKYs. Bilateral PVN microinjection of Ad-CTRP9 significantly decreased the plasma NE in SHRs compared with that of WKYs (Fig. 8)

Discussion

In this study, we investigated the effects of CTRP9 on the hypertensive response, pro-inflammatory cytokines and oxidative stress in the PVN. The novel findings of this study include: (i) bilateral PVN microinjection of Ad-CTRP9 lowers blood pressure and leads to a significant decrease in plasma NE in SHRs; (ii) Ad-CTRP9 attenuates pro-inflammatory cytokines and oxidative stress in the PVN of SHRs; (iii) CTRP9 in PVN ameliorates hypertension may through modulating cytokines and attenuating oxidative stress in spontaneously hypertensive rats.

CTRP9 has multiple beneficial effects against disorders of the cardiovascular system or metabolism[15, 16]. Although CTRP9 has been feted ubiquitously with regard to cardiovascular disorders because of its beneficial effects, the influence of CTRP9 on hypertension has not been studied in depth. Here, we found that CTRP9 was downregulated in spontaneously hypertensive rats, suggesting a negative effect of

CTRP9 on the progression of hypertensin. In this study, we focused on the effects of overexpression CTRP9 on inflammatory cytokines and reactive oxygen species in paraventricular nucleus, and the results showed that CTRP9 decreasd cytokines and attenuated oxidative stress in spontaneously hypertensive rats. Finally, our study on hypertension rats indicated that CTRP9 had a protective role in the development of the progression of hypertension.

It has been well-established that PVN plays a key role in maintaining cardiovascular activity, participating in the regulation of sympathetic nerve activity, and is closely related to the development of hypertension[33]. The excessive sympathetic activity in hypertension is thought to result from high levels of oxidative stress and inflammatory responses in the PVN, and attenuated reactive oxygen species and decreased proinflammatory cytokines improves sympathetic overdrive and lowers blood pressure in hypertensive rats[34, 35]. A rich body of evidence has demonstrated that ROS can stimulate both central and peripheral sympathetic nervous system activity[36]. ROS production in the PVN is reported to be increased in several animal models of hypertension. Inhibition of ROS production in PVN can decrease the RSNA in hypertensive rats[7]. Furthermore, the level of plasma NE (a marker of sympathetic activity) was increased in SHR rats. Bilateral PVN microinjection of Ad-CTRP9 resulted in a substantial decrease in plasma NE, suggesting that CTRP9 can attenuate sympathetic activity in hypertension.

AAV has been used widely in gene therapy because it is efficacious, safe, can be used in different tissues, and has fewer side effects, inflammatory reactions, and immune reactions than those using other vectors. Moreover, AAV can express the target gene continually without DNA integration into the host chromosome[37]. Gene transfection to the hypothalamic paraventricular nucleus (PVN) through bilateral PVN microinjection has been demonstrated to be effective, safe and feasible[38–40]. Therefore, we selected AAV as a vector through bilateral PVN microinjection to overexpress CTRP9 in rats. These features will avoid frequent administration of drugs and improve patient compliance with drug therapy. Our findings may provide a potential target for gene therapy of human hypertension.

Declarations

Conflict of interest

The author declared that he had no conflict of interest.

Funding

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References

1. Cardinale, J.P., et al., Angiotensin II-induced hypertension is modulated by nuclear factor-kappaBin the paraventricular nucleus. Hypertension, 2012. **59**(1): p. 113–21.

2. Davisson, R.L., Physiological genomic analysis of the brain renin-angiotensin system. Am J Physiol Regul Integr Comp Physiol, 2003. **285**(3): p. R498-511.
3. Kang, Y.M., et al., Chronic infusion of enalaprilat into hypothalamic paraventricular nucleus attenuates angiotensin II-induced hypertension and cardiac hypertrophy by restoring neurotransmitters and cytokines. Toxicol Appl Pharmacol, 2014. **274**(3): p. 436–44.
4. Li, H.B., et al., Central blockade of salusin beta attenuates hypertension and hypothalamic inflammation in spontaneously hypertensive rats. Sci Rep, 2015. **5**: p. 11162.
5. Crowley, S.D., The cooperative roles of inflammation and oxidative stress in the pathogenesis of hypertension. Antioxid Redox Signal, 2014. **20**(1): p. 102–20.
6. Pu, J., et al., Cardiomyocyte-expressed farnesoid-X-receptor is a novel apoptosis mediator and contributes to myocardial ischaemia/reperfusion injury. European Heart Journal, 2013. **34**(24): p. 1834–45.
7. Su, Q., et al., Inhibition of reactive oxygen species in hypothalamic paraventricular nucleus attenuates the renin-angiotensin system and proinflammatory cytokines in hypertension. Toxicol Appl Pharmacol, 2014. **276**(2): p. 115–20.
8. Lassegue, B. and R.E. Clempus, Vascular NAD(P)H oxidases: specific features, expression, and regulation. Am J Physiol Regul Integr Comp Physiol, 2003. **285**(2): p. R277-97.
9. Lambeth, J.D., Nox enzymes, ROS, and chronic disease: an example of antagonistic pleiotropy. Free Radic Biol Med, 2007. **43**(3): p. 332–47.
10. Bai, Y., et al., Regional expression of NAD(P)H oxidase and superoxide dismutase in the brain of rats with neurogenic hypertension. Am J Nephrol, 2009. **29**(5): p. 483–92.
11. Wang, G., et al., NADPH oxidase contributes to angiotensin II signaling in the nucleus tractus solitarius. J Neurosci, 2004. **24**(24): p. 5516–24.
12. Yu, X.J., et al., Paraventricular Nucleus Infusion of Oligomeric Proantho Cyanidins Improves Renovascular Hypertension. Front Neurosci, 2021. **15**: p. 642015.
13. Gao, H.L., et al., Oral CoQ10 attenuates high salt-induced hypertension by restoring neurotransmitters and cytokines in the hypothalamic paraventricular nucleus. Sci Rep, 2016. **6**: p. 30301.
14. Si, Y., W. Fan, and L. Sun, A Review of the Relationship Between CTRP Family and Coronary Artery Disease. Curr Atheroscler Rep, 2020. **22**(6): p. 22.
15. Hwang, Y.C., et al., Association of serum C1q/TNF-Related Protein-9 (CTRP9) concentration with visceral adiposity and metabolic syndrome in humans. Int J Obes (Lond), 2014. **38**(9): p. 1207–12.
16. Sun, Y., et al., C1q/tumor necrosis factor-related protein-9, a novel adipocyte-derived cytokine, attenuates adverse remodeling in the ischemic mouse heart via protein kinase A activation. Circulation, 2013. **128**(11 Suppl 1): p. S113-20.
17. Li, Y., et al., CTRP9 Ameliorates Pulmonary Arterial Hypertension Through Attenuating Inflammation and Improving Endothelial Cell Survival and Function. J Cardiovasc Pharmacol, 2016. **67**(5): p. 394–401.

18. Zhou, Q., et al., C1q/TNF-related protein-9 is elevated in hypertension and associated with the occurrence of hypertension-related atherogenesis. Cell Biol Int, 2021. **45**(5): p. 989–1000.
19. Uemura, Y., et al., Adipose-derived factor CTRP9 attenuates vascular smooth muscle cell proliferation and neointimal formation. Faseb Journal, 2013. **27**(1): p. 25–33.
20. Kambara, T., et al., CTRP9 protein protects against myocardial injury following ischemia-reperfusion through AMP-activated protein kinase (AMPK)-dependent mechanism. Journal of Biological Chemistry, 2012. **287**(23): p. 18965–73.
21. Hajjar, R.J., Potential of gene therapy as a treatment for heart failure. Journal of Clinical Investigation, 2013. **123**(1): p. 53–61.
22. Zhu, X., et al., Metalloprotease Adam10 suppresses epilepsy through repression of hippocampal neuroinflammation. J Neuroinflammation, 2018. **15**(1): p. 221.
23. Zheng, L., et al., Rhythmic light flicker rescues hippocampal low gamma and protects ischemic neurons by enhancing presynaptic plasticity. Nat Commun, 2020. **11**(1): p. 3012.
24. Chen, A.D., et al., Angiotensin AT1 receptors in paraventricular nucleus contribute to sympathetic activation and enhanced cardiac sympathetic afferent reflex in renovascular hypertensive rats. Experimental Physiology, 2011. **96**(2): p. 94–103.
25. Sun, W., et al., Oleuropein improves mitochondrial function to attenuate oxidative stress by activating the Nrf2 pathway in the hypothalamic paraventricular nucleus of spontaneously hypertensive rats. Neuropharmacology, 2017. **113**(Pt A): p. 556–566.
26. Kang, Y.M., et al., Brain tumour necrosis factor-alpha modulates neurotransmitters in hypothalamic paraventricular nucleus in heart failure. Cardiovascular Research, 2009. **83**(4): p. 737–46.
27. Li, H.B., et al., Chronic infusion of lisinopril into hypothalamic paraventricular nucleus modulates cytokines and attenuates oxidative stress in rostral ventrolateral medulla in hypertension. Toxicol Appl Pharmacol, 2014. **279**(2): p. 141–9.
28. Agarwal, D., et al., Chronic exercise modulates RAS components and improves balance between pro- and anti-inflammatory cytokines in the brain of SHR. Basic Research in Cardiology, 2011. **106**(6): p. 1069–85.
29. Sun, W., et al., Pomegranate extract decreases oxidative stress and alleviates mitochondrial impairment by activating AMPK-Nrf2 in hypothalamic paraventricular nucleus of spontaneously hypertensive rats. Sci Rep, 2016. **6**: p. 34246.
30. Liu, M., et al., C1q/TNF-related protein-9 promotes macrophage polarization and improves cardiac dysfunction after myocardial infarction. J Cell Physiol, 2019. **234**(10): p. 18731–18747.
31. Gao, H.L., et al., Apigenin Improves Hypertension and Cardiac Hypertrophy Through Modulating NADPH Oxidase-Dependent ROS Generation and Cytokines in Hypothalamic Paraventricular Nucleus. Cardiovascular Toxicology, 2021. **21**(9): p. 721–736.
32. Xu, M.L., et al., Calcitriol ameliorated autonomic dysfunction and hypertension by down-regulating inflammation and oxidative stress in the paraventricular nucleus of SHR. Toxicol Appl Pharmacol, 2020. **394**: p. 114950.

33. Li, H.B., et al., Blockade of Salusin-beta in Hypothalamic Paraventricular Nucleus Attenuates Hypertension and Cardiac Hypertrophy in Salt-induced Hypertensive Rats. J Cardiovasc Pharmacol, 2015. **66**(4): p. 323–31.
34. Wang, M.L., et al., Central blockade of NLRP3 reduces blood pressure via regulating inflammation microenvironment and neurohormonal excitation in salt-induced prehypertensive rats. J Neuroinflammation, 2018. **15**(1): p. 95.
35. Yuan, N., et al., SOD1 gene transfer into paraventricular nucleus attenuates hypertension and sympathetic activity in spontaneously hypertensive rats. Pflugers Arch, 2013. **465**(2): p. 261–70.
36. Campese, V.M., et al., Reactive oxygen species stimulate central and peripheral sympathetic nervous system activity. Am J Physiol Heart Circ Physiol, 2004. **287**(2): p. H695-703.
37. Hadri, L., et al., Therapeutic efficacy of AAV1.SERCA2a in monocrotaline-induced pulmonary arterial hypertension. Circulation, 2013. **128**(5): p. 512–23.
38. Bai, J., et al., Central administration of tert-butylhydroquinone attenuates hypertension via regulating Nrf2 signaling in the hypothalamic paraventricular nucleus of hypertensive rats. Toxicol Appl Pharmacol, 2017. **333**: p. 100–109.
39. Su, Q., et al., 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, 2021. **12**: p. 756671.
40. Huo, C.J., et al., Irisin lowers blood pressure by activating the Nrf2 signaling pathway in the hypothalamic paraventricular nucleus of spontaneously hypertensive rats. Toxicol Appl Pharmacol, 2020. **394**: p. 114953.

Table

Table 1 Rat primers used for real-time RT-PCR.

Rat genes	Forward (5’-3’)	Reverse (5’-3’)
CTRP9	GGCTTCTACTGGTTATGGACGC	GGAGCCTGGATCACCTTTGAT
TNF-α	ACCACGCTCTTCTGTCTACTG	CTT GGTGGTTTGCTACGAC
IL-1β	GCAATGGTCGGGACATAGTT	AGACCTGACTTGGCAGAGGA
IL-6	TCTCTCCGCAAGAGACTTCCA	ATACTGGTCTGTTGTGGGTGG
MCP-1	GTGCTGACCCCAATAAGGAA	TGAGGTGGTTGTGGAAAAGA
$gp91^{phox}$	CTGCCAGTGTGTCGGAATCT	TGTGAATGGCCGTGTGAAGT
$gp47^{phox}$	GGATCACAGAAGGTCCCTAGC	AGAAGTTCAGGGCGTTCACC
GAPDH	AGACAGCCGCATCTTCTTGT	CTTGCCGTGGGTAGAGTCAT

Figures

Fig. 1

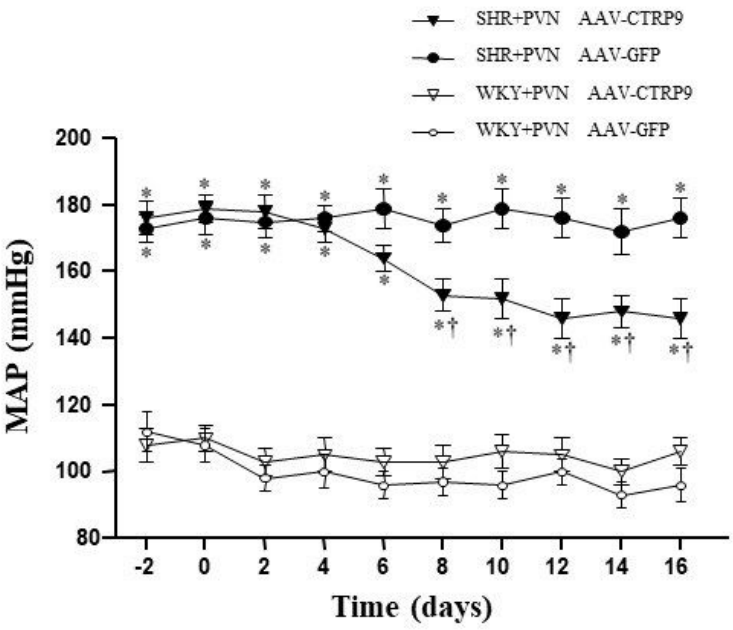

Figure 1

Effect of PVN microinjection of Ad-CTRP9 on mean arterial pressure(MAP) in SHR and WKY rats. The MAP was significantly increased compared with that in WKYs. Bilateral PVN microinjection of Ad-CTRP9 attenuated MAP from day 8 when compared with PVN microinjection of Ad-GFP in SHRs, while still higher than that of the WKY groups. Values are expressed as means ± SE. n = 6 per group; *$P < 0.05$ *vs*. WKY groups (WKY + PVN AAV-CTRP9 or WKY + PVN AAV-GFP); †$P < 0.05$ *vs.* SHR + PVN AAV-CTRP9.

Fig. 2

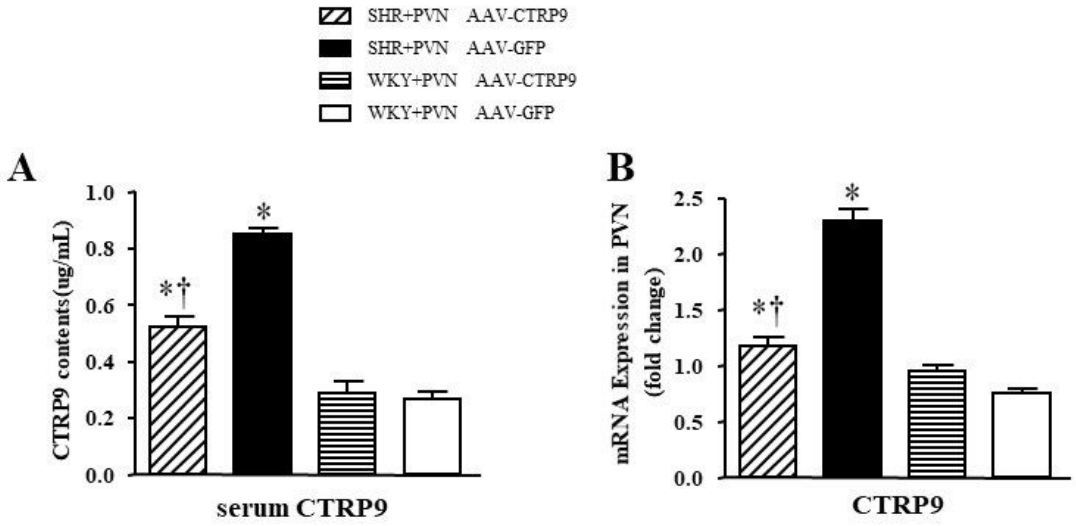

Figure 2

Effect of PVN microinjection of Ad-CTRP9 on serum CTRP9 and mRNA levels of CTRP9 in SHR and WKY rats. (A) CTRP9 concentrations in rat serum are shown. (B) Column diagram analysis of the plasma noradrenaline (NE) in different groups. Values are expressed as means ± SE. n = 6 per group; *$P < 0.05$ *vs*. WKY groups (WKY + PVN AAV-CTRP9 or WKY + PVN AAV-GFP); †$P < 0.05$ *vs*. SHR + PVN AAV-GFP.

Fig. 3

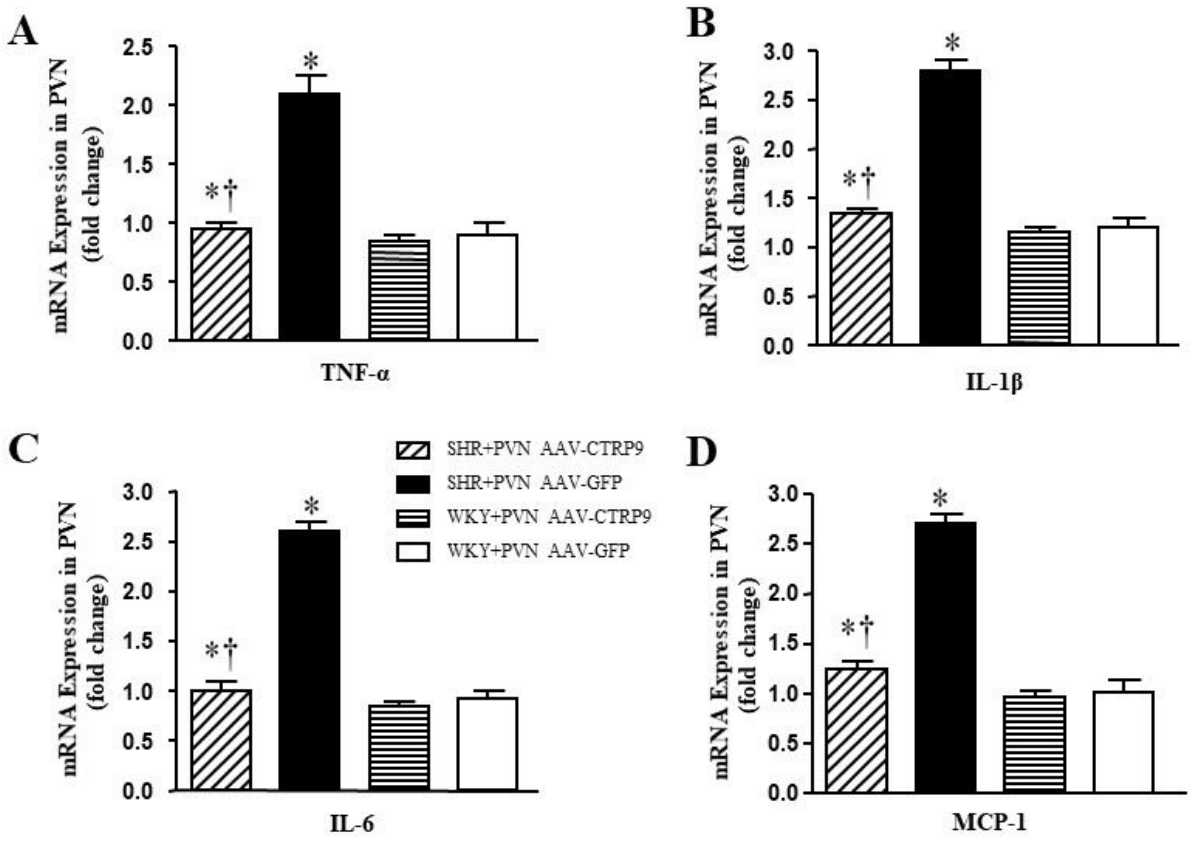

Figure 3

Effect of PVN microinjection of Ad-CTRP9 on mRNA levels of TNF-α, IL-1β, IL-6 and MCP-1 in the PVN. (A) The mRNA expression of TNF-α; (B) The mRNA expression of IL-1β; (C) The mRNA expression of IL-6; and (D) The mRNA expression of MCP-1. Values are expressed as means ± SE. n = 6 per group; *$P < 0.05$ *vs.* WKY groups (WKY + PVN AAV-CTRP9 or WKY + PVN AAV-GFP); †$P < 0.05$ *vs.* SHR + PVN AAV-GFP.

Fig. 4

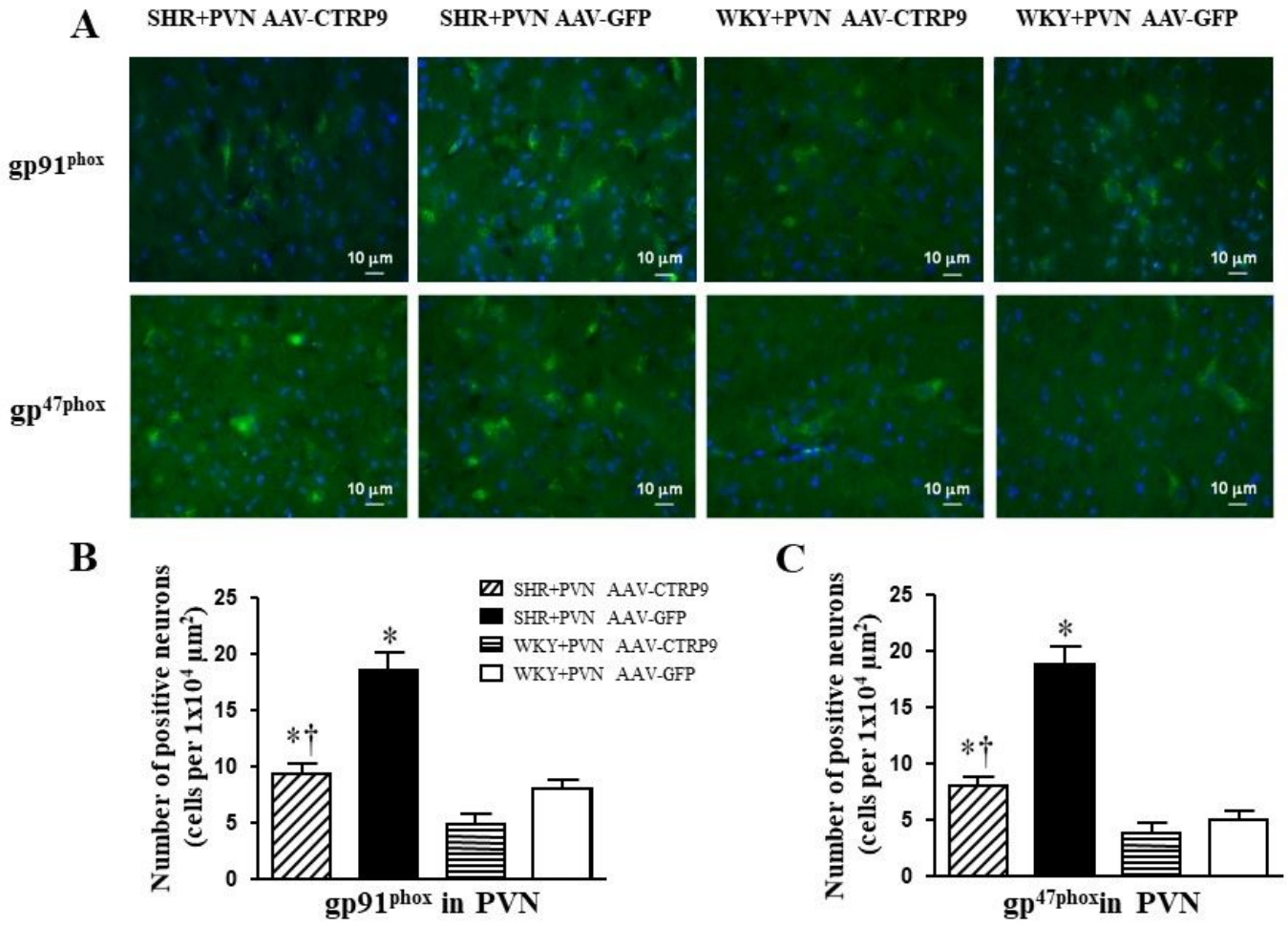

Figure 4

Effect of PVN microinjection of Ad-CTRP9 on gp91^{phox} and gp47^{phox} in the PVN of SHR and WKY rats by immunofluorescence staining. (A) A representative immunofluorescence staining of gp^{91phox} in the PVN of SHR and WKY rats. (B) A representative immunofluorescence image of gp^{47phox} in different groups. Statistical analysis of gp^{91phox} (C) and gp^{47phox} (D) positive neurons in the PVN in SHR and WKY groups. Values are expressed as means ± SE. n = 6 per group; *$P < 0.05$ *vs*. WKY groups (WKY + PVN AAV-CTRP9 or WKY + PVN AAV-GFP); †$P < 0.05$ *vs.* SHR + PVN AAV-GFP.

Fig. 5

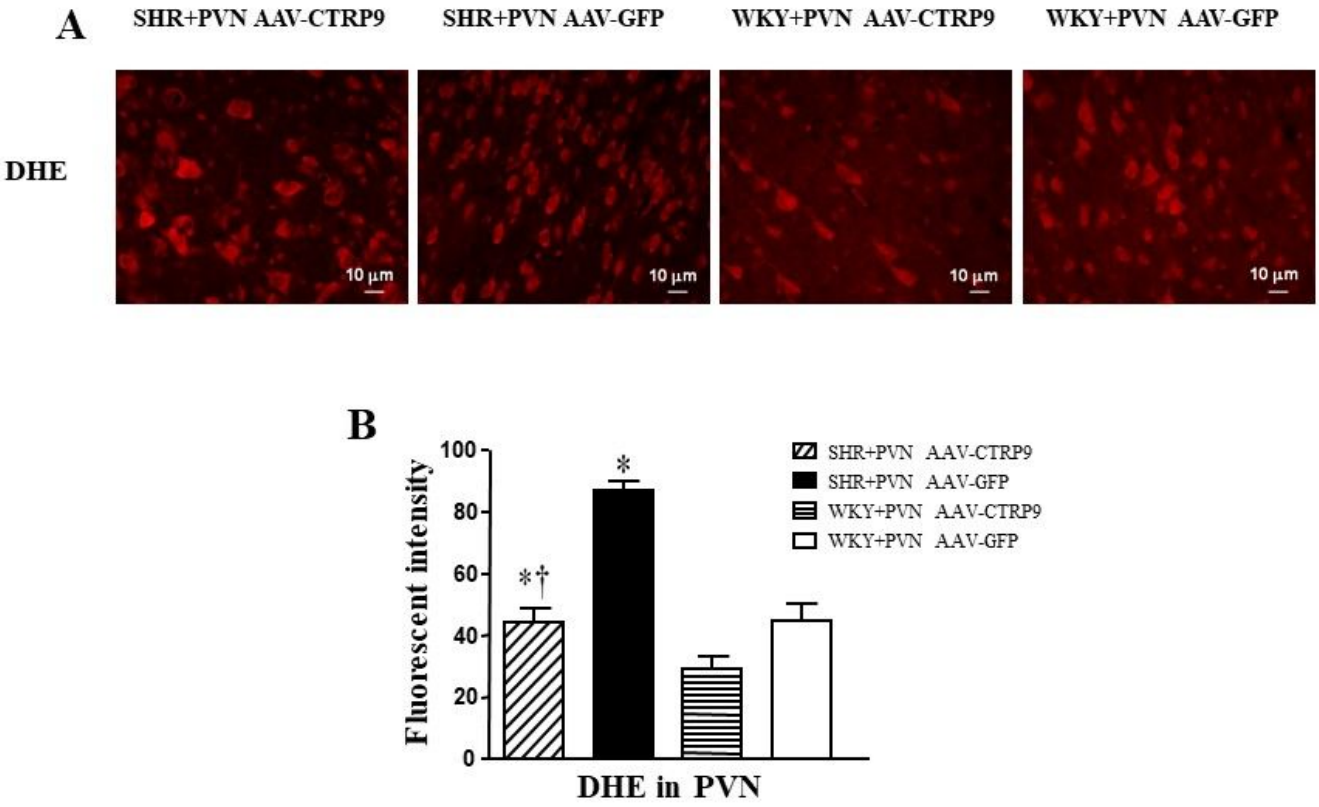

Figure 5

Effect of PVN microinjection of Ad-CTRP9 on DHE in the PVN of SHR and WKY rats. (A) The DHE displayed superoxide anion production (red fluorescence) in the PVN. (B) Relative fluorescence intensity of DHE in the PVN. Values are expressed as means ± SE. n = 6 per group; *$P < 0.05$ *vs*. WKY groups (WKY + PVN AAV-CTRP9 or WKY + PVN AAV-GFP); †$P < 0.05$ *vs.* SHR + PVN AAV-GFP.

Fig. 6

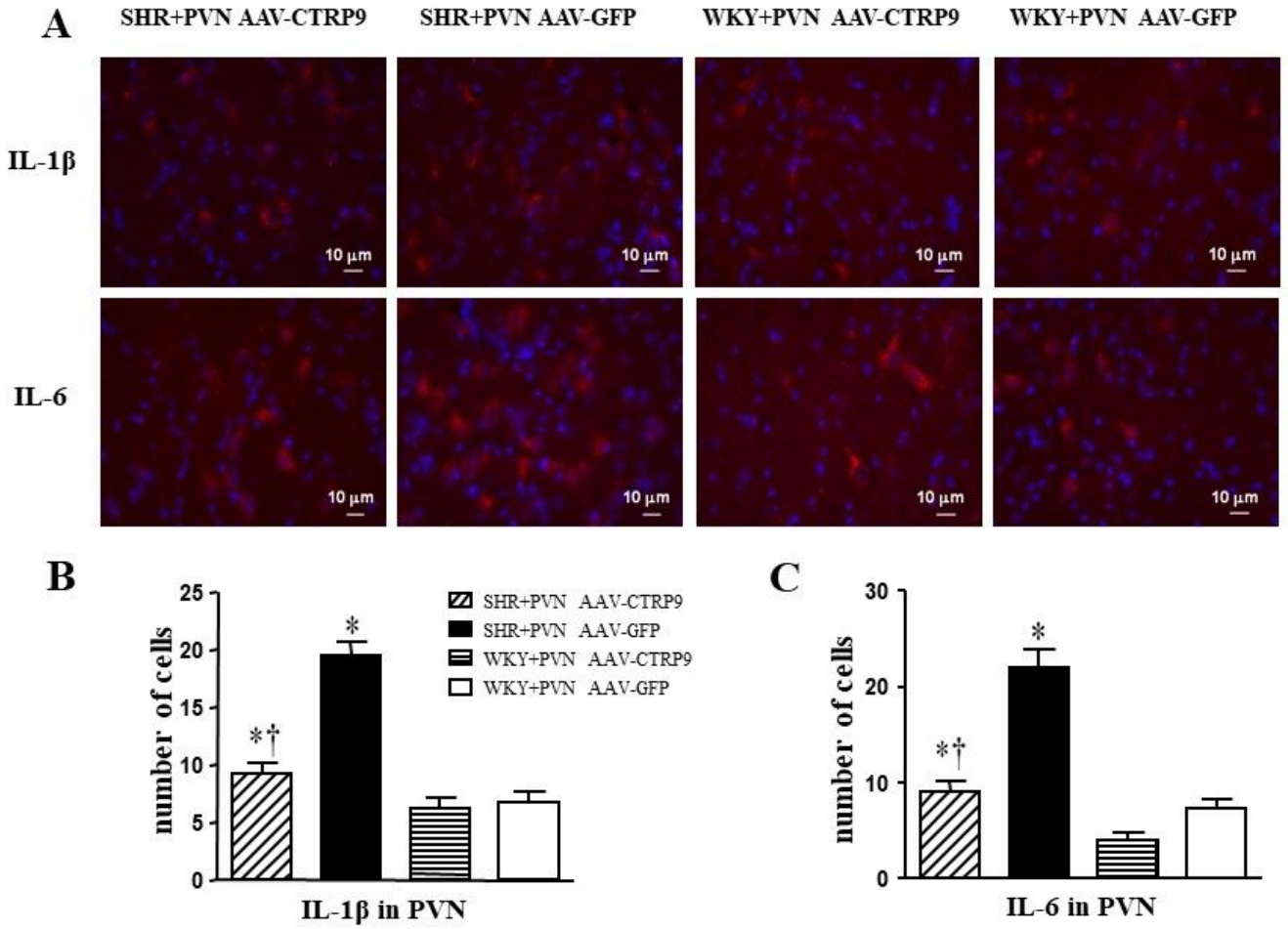

Figure 6

Effect of PVN microinjection of Ad-CTRP9 on IL-1β and IL-6 in the PVN of SHR and WKY rats by immunofluorescence staining. (A) A representative immunofluorescence staining of IL-1βin the PVN of SHR and WKY rats. (B) A representative immunofluorescence image of IL-6 in different groups. Statistical analysis of IL-1β(C) and IL-6 (D) positive neurons in the PVN in SHR and WKY groups. Values are expressed as means ± SE. n = 6 per group; *$P < 0.05$ *vs*. WKY groups (WKY + PVN AAV-CTRP9 or WKY + PVN AAV-GFP); †$P < 0.05$ *vs.* SHR + PVN AAV-GFP.

Fig. 7

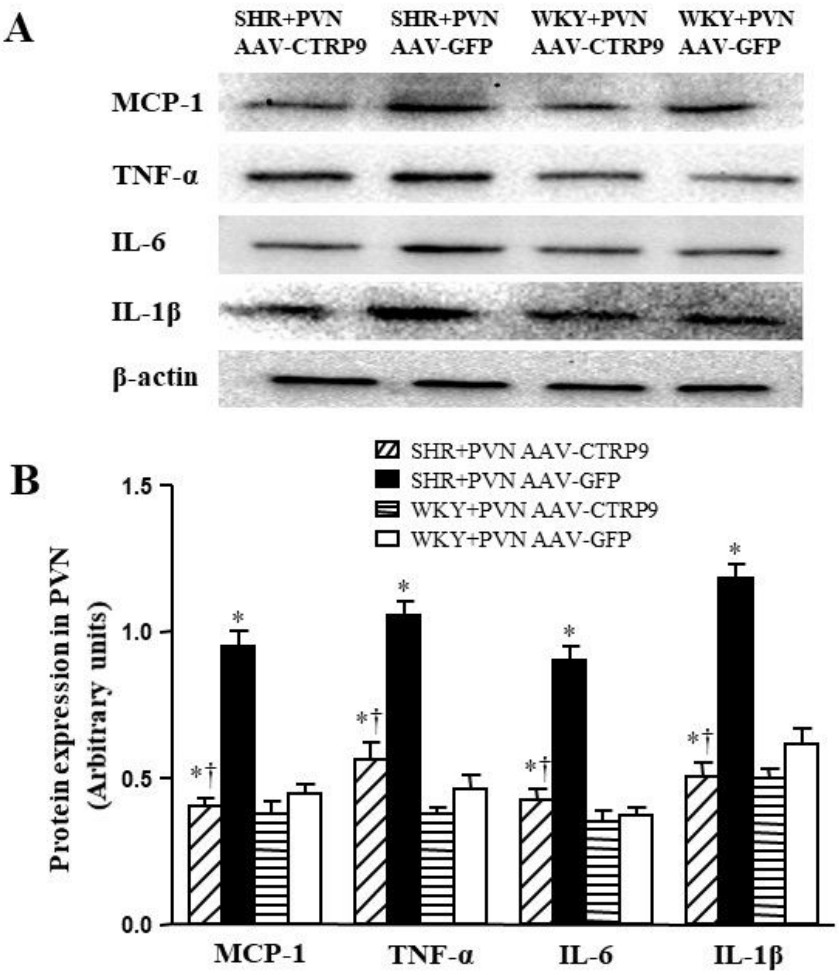

Figure 7

Effect of PVN microinjection of Ad-CTRP9 on protein levels of MCP-1, TNF-α, IL-6 and IL-1β in the PVN of SHR and WKY rats. (A) A representative immunoblot; and (B) densitometric analysis of protein expression of MCP-1, TNF-α, IL-6, IL-1β and β-actin in different groups. Values are expressed as means ± SE. n = 6 per group; *$P < 0.05$ *vs*. WKY groups (WKY + PVN AAV-CTRP9 or WKY + PVN AAV-GFP); †$P < 0.05$ *vs.* SHR + PVN AAV-GFP.

Fig. 8

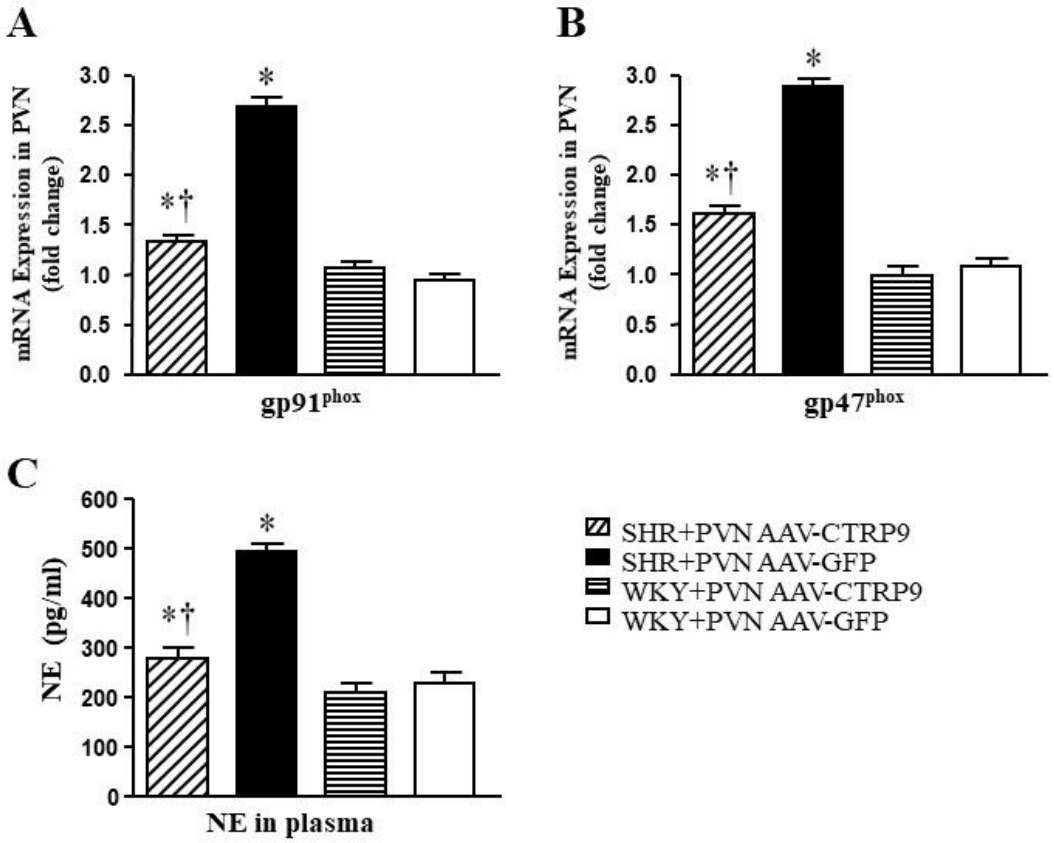

Figure 8

Effect of PVN microinjection of Ad-CTRP9 on mRNA levels of gp91^{phox} and gp47^{phox}, and plasma noradrenaline (NE) in SHR and WKY rats. (A) The mRNA expression of gp91^{phox}; (B) The mRNA expression of gp47^{phox}. (C) Column diagram analysis of the plasma NE in different groups. Values are expressed as means ± SE. n = 6 per group; *$P < 0.05$ *vs*. WKY groups (WKY + PVN AAV-CTRP9 or WKY + PVN AAV-GFP); †$P < 0.05$ *vs.* SHR + PVN AAV-GFP.