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Central administration of *tert*-butylhydroquinone attenuates hypertension via regulating Nrf2 signaling in the hypothalamic paraventricular nucleus of hypertensive rats

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ABSTRACT

Reactive oxygen species (ROS) in the paraventricular nucleus (PVN) play a pivotal role in the pathogenesis of hypertension. Nuclear factor E2-related factor-2 (Nrf2) is an important transcription factor that modulates cell antioxidant defense response against oxidative stress. The present study aimed to explore the efficacy of PVN administration of *tert*-butylhydroquinone (tBHQ), a selective Nrf2 activator, in hypertensive rats. 16-week-old spontaneously hypertensive rats (SHR) and normotensive Wistar-Kyoto (WKY) rats were used in this study. These rats were chronic bilateral PVN infusion of tBHQ (0.8 μg/day), or oxygen free radical scavenger tempol (20 μg/h), or vehicle for 2 weeks. SHR rats had higher mean arterial pressure (MAP), plasma norepinephrine (NE) levels, and sympathetic nerve activity (RSNA) and lower PVN levels of Nrf2, hemeoxygenase-1 (HO-1), superoxide dismutase-1 (SOD1) and catalase (CAT) as compared with those in the WKY group. Bilateral PVN infusion of tBHQ or tempol significantly reduced MAP, RSNA, plasma NE levels in SHR rats. In addition, tBHQ treatment enhanced the nuclear accumulation of Nrf2 and increased the expression of HO-1, CAT and SOD1 in SHR rats. Furthermore, tBHQ attenuated PVN levels of ROS, the expression of proinflammatory cytokines and restored the imbalance of neurotransmitters in PVN. Knockdown of Nrf2 in the PVN by adeno-associated virus mediated small interfering RNA abrogated the protective effects of tBHQ on hypertension. These findings suggest that PVN administration of tBHQ can attenuate hypertension by activation of the Nrf2-mediated signaling pathway.

1. Introduction

Sympathoexcitation is one of the key factors that contribute to the pathogenesis of hypertension. The hypothalamic paraventricular nucleus (PVN) has been regarded as one of the most important region that can influence the sympathetic outflow in the central nervous system (Patel, 2000; Gabor and Leenen, 2012). Previous studies have shown that reactive oxygen species (ROS) and proinflammatory cytokines (PICs) in the PVN contribute to sympathoexcitation in different kinds of hypertension (Oliveira-Sales et al., 2009; Su et al., 2014; Dange et al., 2015; Xue et al., 2016). Our recent studies suggest that inhibition of reactive oxygen species in PVN can attenuate PICs and renin–angiotensin system (RAS), restore the imbalance between inhibitory and excitatory neurotransmitters, and thereby reduce sympathetic activity and blood pressure (Su et al., 2016).

The Nuclear factor E2 related factor-2 (Nrf2) is an important transcription factor that can modulate cell antioxidant defense response against oxidative stress by regulating the synthesis of a range of antioxidants and detoxification enzymes (Barancik et al., 2016). Under normal conditions, Nrf2 binds to its inhibitor Kelch-like ECH-associated inhibitor 1 (Keap1). This Keap1/Nrf2 complex is targeted for proteasomal degradation. When stimulated by stress, the conformation of Keap1/Nrf2 complex changes, resulting in the increase in nuclear translocation of the activated Nrf2. Then, Nrf2 binds to a group of antioxidant response element (ARE)-dependent cytoprotective genes, including hemeoxy-genase-1 (HO-1), NAD(P)H: quinone oxidoreductase-1 (NQO1) and glutathione S-transferasea-1 (GST-a1). Recently, activation of the Nrf2-ARE pathway has been documented to be

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beneficial in many cardiovascular diseases (Barancik et al., 2016). A previous study has shown that reduction in nuclear translocation of Nrf2 in the rostral ventrolateral medulla (RVLM) neurons contributes to hypertension induced by LPS-mediated systemic inflammation (Wu et al., 2016). Our group has found that oral administration of oleuropein can reduce oxidative stress and improve mitochondrial function in PVN by activation of the Nrf2-mediated signaling pathway (Sun et al., 2016a). Despite the positive roles of Nrf2 in preventing oxidative stress, there is little direct evidence that activation of the Nrf2-mediated signaling pathway in PVN has protective effects against hypertension. The underlying mechanisms of its potential protective effects on hypertension remain to be fully elucidated.

Tert-butylhydroquinone (tBHQ), a synthetic phenolic antioxidant, is widely used as a selective Nrf2 activator. Growing evidence has confirmed its antioxidant activity in different kinds of diseases (Li et al., 2014b; Wang et al., 2015; Wu et al., 2015; Duan et al., 2016; Ye et al., 2016). Notably, a recent study indicates that oral administration of tBHQ can lower blood pressure in hypertensive mice (Xu et al., 2016). However, the precise mechanisms by which it protects against hypertension remains unclear. In the present study, we investigated the effects of PVN infusion of tBHQ on hypertension and Nrf2 signaling pathway. We also determined whether ROS, PICs and neurotransmitters in the PVN were involved in the effects of tBHQ.

2. Materials and methods

2.1. Animals

Male spontaneously hypertensive rats (SHR) and Wistar-Kyoto rats (WKY) weighing 250 g–270 g were purchased from Charles River Laboratory Animal, Ltd. (Beijing, China). The rats were housed individually in a room with controlled temperature (20–23 °C) and light: dark cycle (12 h: 12 h). All animals were allowed access to standard chow and tap water *ad libitum*. All of the animal procedures were approved by the Animal Care and Use Committees of Xi'an Jiaotong University and conducted in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

2.2. Drug and preparation

tBHQ was purchased from Sigma-Aldrich and first dissolved in 100% dimethyl sulfoxide (DMSO) as a stock. Then artificial cerebrospinal fluid (aCSF) was added to prepare for the working solution with a final concentration of 1.8 mM in aCSF with 1% DMSO. Oxygen free radical scavenger tempol (Sigma) was dissolved in aCSF. The osmotic minipumps (ALZET, model 1004; infusion rate of 0.11 μl/h) were then loaded with fresh tBHQ solution or tempol according to the manufacturer's protocols. The vehicle solution was a matching concentration of DMSO (1%) in aCSF. In general, the rats were PVN infusion of tBHQ (0.8 μg/day), or tempol (20 μg/h), or vehicle for two weeks. The doses used in this study were based on previous effective studies (Shih et al., 2005; Su et al., 2014) and our preliminary experiment results.

2.3. Experimental design

Experimental protocol 1: After anesthesia, cannulae were implanted bilaterally into the PVN (1.8 mm caudal to the bregma, 0.4 mm lateral to central line, and 7.8 mm below the skull surface) using brain stereotaxic apparatus, as described previously (Li et al., 2015b). Seven days after surgical recovery, the osmotic minipumps were then implanted subcutaneously and connected to the PVN cannulae for drug administration of tBHQ or tempol for 2 weeks. At the end of the second week, rats were anesthetized for the sympathetic nerve activity (RSNA) measurement and then euthanized to collect blood and tissue samples for further analysis. All the precise injection sites were verified by histological examination. The success rate of bilateral PVN microinjecton was about 70%. Animals with verifiable bilateral PVN injection sites were used for the final analysis.

Experimental protocol 2: Adeno-associated virus mediated small interfering RNA against Nrf2 (AAV-NRF2-siRNA) or control vectors (AAV-SCM-siRNA) with enhanced green fluorescent protein (eGFP) were purchased from Hanbio Biotechnology Co., Ltd. (Shanghai, China). The siRNA sequence for Nrf2 is 5′-GTCTTCAGCATGTTACGTGATGAGGATGG-3′ (Wruck et al., 2007). The intra-PVN infusion of AAV was conducted as previously described (Chen et al., 2014). Briefly, rats were anesthetized by intraperitoneal injection of a ketamine (90 mg/kg) and xylazine (10 mg/kg) mixture; the cannulae were implanted bilaterally into the PVN. Then they were connected to two 10 μl Hamilton micro-syringes which were mounted on an infusion pump. The viral vectors (1 μl of 1×10^{12} genomic particles/ml) were infused at a rate of 0.1 μl/min for 10 min. Two weeks after AAV injection, the osmotic minipumps were then implanted subcutaneously and connected to the PVN cannulae for tBHQ (0.8 μg/day) administration for another 2 weeks. The SHR rats were randomly divided into four groups: (i) AAV-SCM-siRNA + vehicle; (ii) AAV-SCM-siRNA + tBHQ; (iii) AAV-NRF2-siRNA + vehicle; (iv) AAV-NRF2-siRNA + tBHQ. At the end of the experiment, tissue samples were collected for further analysis.

2.4. Measurement of mean arterial pressure (MAP)

Blood pressure (BP) was measured by using a noninvasive tail-cuff system (NIBP, AD Instruments, Australia) in conscious rats as previously described (Sun et al., 2016b). In order to diminish the stress-induced BP fluctuations, the rats were trained by measuring MAP daily for at least 7 days before the operation. The MAP values were averaged from five measurements obtained from each rat.

2.5. Sympathetic neural recordings

Under anesthesia with a ketamine (90 mg/kg) and xylazine (10 mg/kg) mixture (ip), the left renal nerves of rat were isolated for recording sympathetic nerve activity (RSNA). The rectified and integrated RSNA were recorded using methods described as previously (Kang et al., 2009; Li et al., 2014a).

2.6. Collection of tissue samples

The bilateral PVN tissue was isolated according to Palkovits's microdissection procedure as previously described (Kang et al., 2011). Tissue samples were then stored at − 80 °C for future analysis.

2.7. Immunohistochemical and immunofluorescence studies

The PVN immunohistochemical and immunofluorescence staining were conducted as previously described (Qi et al., 2016). We used the following antibodies: rabbit anti-Nrf2 (cat# ab31163, Abcam), rabbit anti-SOD1 (cat#ab16831, Abcam), rabbit anti-NOX2 (cat#ab-31092, Abcam), rabbit anti-Fra-LI (cat#sc253, Santa Cruz), goat anti-IL-1β (cat#sc-1251, Santa Cruz),goat anti-GAD67 (cat#sc-7512, Santa Cruz), mouse anti-TH (cat#sc-25269, Santa Cruz), rabbit anti-HO-1 (cat#bs-2075R, Bioss), Immunofluorescent staining for Nrf2 was observed with a confocal laser-scanning microscope (Zeiss LSM 710, Carl Zeiss, Inc.). Other immunohistochemistry or immunofluorescent stained sections were photographed with a conventional light microscopy (DP70, Olympus, Tokyo, Japan). For each animal, the numbers of positive staining cells in the bilateral PVN were manually counted in three consecutive sections and the average value was used.