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Endogenous hydrogen peroxide in the hypothalamic paraventricular nucleus regulates neurohormonal excitation in high salt-induced hypertension

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HIGHLIGHTS

- Salt-induced hypertensive rats exhibit neurohormonal excitation in the PVN.
- PVN PEG-CAT attenuates neurohormonal excitation and expression of RAS in hypertension.
- PVN PEG-CAT attenuates imbalances of neurotransmitters and cytokines in hypertension.
- PVN ATZ augments hypertension-induced neurohormonal excitation and expression of RAS.
- PVN ATZ augments hypertension-induced imbalances of neurotransmitters and cytokines.

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ABSTRACT

Reactive oxygen species (ROS) in the brain plays an important role in the progression of hypertension and hydrogen peroxide (H_2O_2) is a major component of ROS. The aim of this study is to explore whether endogenous H_2O_2 changed by polyethylene glycol-catalase (PEG-CAT) and aminotriazole (ATZ) in the hypothalamic paraventricular nucleus (PVN) regulates neurotransmitters, renin-angiotensin system (RAS), and cytokines, and whether subsequently affects the renal sympathetic nerve activity (RSNA) and mean arterial pressure (MAP) in high salt-induced hypertension. Male Sprague–Dawley rats received a high-salt diet (HS, 8% NaCl) or a normal-salt diet (NS, 0.3% NaCl) for 10 weeks. Then rats were treated with bilateral PVN microinjection of PEG-CAT (0.2 i.u./50 nl), an analog of endogenous catalase, the catalase inhibitor ATZ (10 nmol/50 nl) or vehicle. High salt-fed rats had significantly increased MAP, RSNA, plasma norepinephrine (NE) and pro-inflammatory cytokines (PICs). In addition, rats with high-salt diet had higher levels of NOX-2, NOX-4 (subunits of NAD(P)H oxidase), angiotensin-converting enzyme (ACE), interleukin-1beta (IL-1β), glutamate and NE, and lower levels of gamma-aminobutyric acid (GABA) and interleukin-10 (IL-10) in the PVN than normal diet rats. Bilateral PVN microinjection of PEG-CAT attenuated the levels of RAS and restored the balance of neurotransmitters and cytokines, while microinjection of ATZ into the PVN augmented those changes occurring in hypertensive rats. Our findings demonstrate that ROS component H_2O_2 in the PVN regulating MAP and RSNA are partly due to modulate neurotransmitters, renin-angiotensin system, and cytokines within the PVN in salt-induced hypertension.

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1. Introduction

Hypertension has become a serious public-health problem and social concern. An excess of dietary salt is listed as one of the major common environmental factors, which significantly contributes to the development of hypertension (Frohlich and Varagic, 2005; Ha, 2014). Although the underlying molecular and cellular mechanisms of high salt-induced hypertension remain unclear, considerable evidence has showed that the development of cardiovascular diseases is associated with neurohormonal excitation in the hypothalamic paraventricular nucleus (PVN) (Su et al., 2014; Yu et al., 2013). The PVN is a principal cardiovascular center modulating the mean arterial pressure (MAP) and renal sympathetic nerve activity (RSNA) in hypertension (Cardinale et al., 2012; Kang et al., 2014). Recent evidence suggests that reactive oxygen species (ROS) plays a critical role in the regulation of sympathoexcitation and hypertensive response in the PVN (Crowley, 2014; Pu et al., 2013; Su et al., 2014). High salt intake leads to an increased generation of ROS, and the activation of oxidative stress contribute to the progression of hypertension (Ando, 2014; Wilcox, 2005). ROS contains superoxide anion ($O_2^{\bullet}-$), hydrogen peroxide (H_2O_2), and hydroxyl radical ($^{\bullet}OH$). Our recent studies have demonstrated that hypertensive rats produced excessive amounts of ROS, such as superoxide ($O_2^{\bullet}-$) (Wilcox, 2005). Endogenous or exogenous chemicals including H_2O_2, capsaicin, and adenosine, contribute to increase blood pressure by activating the sympathetic nervous system (Du and Chen, 2007; Guo and Moazzami, 2004; Malliani and Montano, 2002). The studies demonstrated that neuronal activity is influenced by the balance of various neurotransmitters activities, including glutamate, norepinephrine (NE), and gamma-aminobutyric acid (GABA) (Kang et al., 2011a; Kang et al., 2011b). Decreased inhibitory neurotransmitters, GABA and increased excitatory adrenergic and glutamatergic in the PVN are involved in sympathetic regulation in hypertension (Agarwal et al., 2011; Horn et al., 1998). In this study, we explored the impact of endogenous H_2O_2 in the PVN on neurotransmitters levels.

The pro-inflammatory cytokines (PICs) levels, such as tumor necrosis factor-alpha (TNF-α), interleukin-1beta (IL-1β), interleukin-6 (IL-6) and anti-inflammatory cytokines (AICs), such as interleukin-10 (IL-10), have been proven to play a central role in the pathogenesis of hypertension (Agarwal et al., 2013; Cardinale et al., 2012). Angiotensin II (Ang II) infusion increased RAS activation in the PVN, contributing to sympathoexcitation and hypertensive response (Sriramula et al., 2011; Su et al., 2014). Our recent findings suggest RAS inhibition has been found to restore the balance between pro- and anti-inflammatory cytokines in the PVN, and thereby contributes to sympathetic activity and hypertension in the Ang II-induced hypertension (Kang et al., 2014). Another study showed that cytokine mediators are capable of regulating different kinds of RAS components in the PVN (Kang et al., 2008; Sriramula et al., 2013). However, it is still unclear whether ROS components H_2O_2 modulating cytokines balance and the levels of RAS in the PVN is closely associated with the central mechanisms of salt-induced hypertension.

The aim of this study was undertaken to explore endogenous H_2O_2 impacts the expression of angiotensin-converting enzyme (ACE), regulates the balance between the excitatory and inhibitory neurotransmitters and the balance between pro- and anti-inflammatory cytokines, and contributes to mediate sympathetic activity and blood pressure in the PVN in high salt-induced hypertensive rats. The results from this study may contribute to a better understanding of the disease process and help in designing new strategies for the treatment of hypertension.

2. Materials and methods

2.1. Animals

Adult male Sprague–Dawley rats weighing 250–275 g were housed in the right temperature and light-controlled (23 ± 2 °C, 12 h light/dark cycle, respectively) animal quarters. They were fed rat chow and tap water *ad libitum*. All the procedures were approved by the Experimental Animal Care Use Committees of Xi'an Jiaotong University. All the experiments conformed to the *Guide for the Care and Use of Laboratory Animals* published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996).

2.2. General experimental protocol

During the 10-week experimental period, male SD rats were fed different rat chow, which were purchased from Experimental Animal Center of Xi'an Jiaotong University Health Science Center. The rats received a high-salt diet (HS, 8% NaCl) and normal-salt diet (NS, 0.3% NaCl). The systolic blood pressure of rats were measured weekly by the tail-cuff method using an automatic sphygmomanometer. At the end of the 10th week, rats were anaesthetized with a ketamine (80 mg/kg) and xylazine (10 mg/kg) mixture *via* intraperitoneal injection (ip). The bilateral PVN microinjections were carried out with polyethylene glycol-catalase (PEG-CAT, 0.2 i.u./50 nl), the catalase inhibitor aminotriazole (ATZ, 10 nmol/50 nl), or vehicle (artificial cerebrospinal fluid, aCSF) in different groups. PEG-CAT and ATZ were purchased from Sigma Chemical. Artificial cerebrospinal fluid was obtained from Harvard Apparatus. The doses used in this study were determined based on previous reports (Xu et al., 2011b; Yu et al., 2007).

2.3. Model of high salt-induced hypertension

Sprague–Dawley (SD) rats induce hypertension with a high-salt diet using methods described as previously (Gu et al., 2008; Ogihara et al., 2001). SD rats weighing 250–275 g were used in this study. The HS rats were fed with a high-salt diet (8% NaCl), and the NS rats were fed with a normal-salt diet (0.3% NaCl) for 10 weeks. High-salt diet rats increase water intake and urinary volume output when compared with normal-salt diet rats.

2.4. Bilateral PVN microinjection

The experiment of bilateral PVN microinjection was conducted on rats after 10 weeks on a high-salt diet or normal-salt diet. The method was used to inject bilateral PVN as described previously (Francis et al., 2000; Li et al., 2014). Briefly, under anesthesia, the head was placed into a stereotaxic apparatus. The location was 1.8 mm caudal to the bregma, 0.4 mm lateral to central line, and 7.9 mm below the skull surface (Chen et al., 2011; Zhu et al., 2004). The skull was then opened, and a glass microelectrode was implanted into the PVN according to stereotaxic coordinates. 50 nl of PEG-CAT, ATZ, or vehicle were microinjection into the bilateral PVN each side, which were completed within 1 min. Rats received buprenorphine (0.01 mg/kg, sc) immediately following surgery. The success rate of bilateral PVN microinjection was 68%, and only animals with verifiable bilateral PVN injection sites were used in the final analysis.

2.5. Mean arterial pressure (MAP) measurement

Arterial pressure of the rats were measured noninvasively weekly by the tail-cuff method using an automatic sphygmomanometer. The blood pressure and heart rate were measured as