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Chronic infusion of epigallocatechin-3-*O*-gallate into the hypothalamic paraventricular nucleus attenuates hypertension and sympathoexcitation by restoring neurotransmitters and cytokines

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HIGHLIGHTS

- PVN infusion of EGCG in spontaneously hypertensive rats is reported.
- PVN infusion of EGCG attenuates hypertension and sympathoexcitation.
- PVN infusion of EGCG attenuates PVN oxidative stress and NF-κB activity.
- PVN infusion of EGCG restores hypertension-induced imbalance of cytokines.
- PVN infusion of EGCG restores hypertension-induced imbalance of neurotransmitters.

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ABSTRACT

Reactive oxygen species (ROS) in the brain are involved in the pathogenesis of hypertension. Epigallocatechin-3-*O*-gallate (EGCG), one of the active compounds in green tea, has anti-oxidant, anti-inflammatory and vascular protective properties. This study was designed to determine whether chronic infusion of EGCG into the hypothalamic paraventricular nucleus (PVN) attenuates ROS and sympathetic activity and delays the progression of hypertension by up-regulating anti-inflammatory cytokines, reducing pro-inflammatory cytokines (PICs) and decreasing nuclear factor-kappa B (NF-κB) activity, as well as restoring the neurotransmitters balance in the PVN of spontaneously hypertensive rats (SHR). Adult normotensive Wistar–Kyoto (WKY) rats and SHR received bilateral PVN infusion of EGCG (20 μg/h) or vehicle *via* osmotic minipumps for 4 weeks. SHR showed higher mean arterial pressure, plasma proinflammatory cytokines and circulating norepinephrine (NE) levels compared with WKY rats. SHR also had higher PVN levels of the subunit of NAD(P)H oxidase ($gp91^{phox}$), ROS, tyrosine hydroxylase, and PICs; increased NF-κB activity; and lower PVN levels of interleukin-10 (IL-10) and 67 kDa isoform of glutamate decarboxylase (GAD67) than WKY rats. PVN infusion of EGCG attenuated all these changes in SHR. These findings suggest that SHR have an imbalance between excitatory and inhibitory neurotransmitters, as well as an imbalance between pro- and anti-inflammatory cytokines in the PVN. Chronic inhibition of ROS in the PVN restores the balance of neurotransmitters and cytokines in the PVN, thereby attenuating hypertensive response and sympathetic activity.

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1. Introduction

Hypertension is a major factor responsible for deaths caused by cardiovascular diseases. Hypertension is characterized by chronic inflammation, increased reactive oxygen species (ROS) and elevated blood pressure (BP) and sympathetic tone (Li et al., 2015a, 2014). Although the brain has typically been considered as a target for late stage hypertensive disease, a growing body of evidence has implicated the role of the brain in the initiation of all forms of hypertension including essential hypertension (Chalmers, 1998; de Wardener, 2001). The hypothalamic paraventricular nucleus (PVN) is a predominant region coordinating nervous signals for maintaining resting BP and sympathetic tone and contains excitatory and inhibitory neurotransmitters, which exert their actions to coordinate autonomic and neuroendocrine homeostasis (Davisson et al., 2000; Sriramula et al., 2008). Previous studies have also shown that the enhanced sympathoexcitation is due to an increase in excitatory adrenergic and glutamatergic activities and a decrease in GABA ergic activity in the PVN (Kang et al., 2009a, 2014).

Hypertension is a chronic low-grade inflammation. In the last few years, research has implicated brain cytokines in the pathogenesis of hypertension. The pro-inflammatory cytokines (PICs) such as tumour necrosis factor-alpha (TNF-α), interleukin-1β (IL-1β) and IL-6 in the PVN act as neuromodulators and play a pivotal role in the sympathetic regulation of BP (Granger, 2006). Furthermore, we and others have demonstrated that elevated cytokines in the PVN induce the production of ROS (Kang et al., 2009b; Sriramula and Francis, 2015). ROS, produced in the neurons of the brain, in turn, are cytotoxic, further perpetuating sympathoexcitatory effects (Su et al., 2014). It has also been suggested that ROS production *via* NADPH oxidases is associated with increased levels of PICs, thereby accelerating the progression of hypertension (Su et al., 2014, 2016). However, the potential implications of the chronic infusion of a ROS inhibitor in the progression of hypertension are not clear at the molecular level.

Green tea is a widely consumed beverage worldwide. Polyphenols, also known as catechins, are the major compounds found in green tea; of these polyphenols, epigallocatechin-3-*O*-gallate (EGCG) is the most abundant accounting for 50%–80% of total phenols found on green tea (Liu et al., 2013; Wolfram, 2007). EGCG has been a research focus in recent years due to its high antioxidant activity and anti-inflammatory properties (Byun et al., 2014; Jeong et al., 2004; Yue et al., 2000). Studies have shown that EGCG was effective in protecting cultured retinal ganglion cells (RGC) against H_2O_2-induced oxidative-stress injury by attenuating intracellular ROS generation (Jin et al., 2015). In addition, EGCG has been shown to have some protective properties in the cardiovascular system (Cabrera et al., 2006; Hao et al., 2007). However, the effects of EGCG on delaying the progression of hypertension have not yet been elucidated, and there is very little knowledge about the underlying molecular mechanisms. The present study examined the effects of chronic EGCG infusion in the PVN on blood pressure and sympathoexcitation. We also explored the impact of chronic infusion with EGCG on oxidative stress, NF-κB activity, anti- and pro-inflammatory cytokines, and neurotransmitters in the PVN of spontaneously hypertensive rats (SHR).

2. Materials and methods

2.1. Animals

Experiments were conducted with 14-week old male normotensive Wistar–Kyoto (WKY) rats and SHR weighing from 275 g to 300 g. All 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 animal and experimental procedures in this study were reviewed and approved by the Animal Care and Use Committees of Xi'an Jiaotong University and were conducted in accordance with the Guide for the Care and Use of Laboratory Animals (National Institutes of Health publication No. 85-23, revised 1996).

2.2. General experimental protocol

Rats were anesthetized with a ketamine (80 mg/kg) and xylazine (10 mg/kg) mixture (ip) and bilateral PVN cannulae were implanted. The osmotic minipumps (Alzet Model 1004, Durect Corporation, Cupertino, CA) were implanted subcutaneously and connected to the PVN cannulae for the continuous infusion of epigallocatechin-3-*O*-gallate (EGCG) or vehicle (artificial cerebrospinal fluid, aCSF) for 4 weeks directly into the bilateral PVN (Kang et al., 2009b; Su et al., 2014). Minipumps were connected to the bilateral PVN cannulae for continuous infusion of EGCG, which inhibits the production of ROS, at a total dose of 20 μg/h. The doses used in this study were based on previous reports(Yi et al., 2015). The rats were divided into four groups: (n = 20/group): (i) WKY + PVN vehicle; (ii) WKY + PVN EGCG; (iii) SHR + PVN vehicle; and (iv) SHR + PVN EGCG. At the end of 4 weeks, the rats were anesthetized with a ketamine (80 mg/kg) and xylazine (10 mg/kg) mixture (ip) and euthanized to collect blood and brain tissue for molecular and immunohistochemical analyses.

2.3. Bilateral PVN cannulae implantation for chronic infusion studies

The method for implantation of bilateral PVN cannulae has been described previously (Kang et al., 2009b; Li et al., 2015b; Su et al., 2014). Briefly, rats were anaesthetized with a ketamine (80 mg/kg) and xylazine (10 mg/kg) mixture (ip) and then placed into a stereotaxic apparatus. A skin incision was made, the skull was opened, and the dura was dissected parallel to the sinus vein. A stainless steel double cannula was implanted into the PVN (1.8 mm posterior, 0.4 mm lateral to the bregma, and 7.9 mm ventral to the zero level) according to the stereotaxic coordinates. The cannula was fixed to the cranium using dental acrylic and two stainless steel screws. Rats received buprenorphine (0.01 mg/kg, sc) immediately following surgery and 12 h postoperatively for pain relief. The success rate of bilateral PVN cannulation is 68%, and only animals with verifiable bilateral PVN injection sites were selected for the final analysis.

2.4. Measurement of mean arterial pressure (MAP)

Blood pressure was determined by a tail-cuff occlusion method. This method and subsequent data analysis have been described previously (Kang et al., 2014; Li et al., 2014). Briefly, unanesthetized rats were warmed to an ambient temperature of 32 °C by placing the rats in a holding device mounted on a thermostatically controlled warming plate (NIBP, ADInstruments, Australia). Rats were allowed to habituate to this procedure for 3 days prior to each experiment. Blood pressure values were averaged from seven consecutive cycles per day obtained from each rat.

2.5. Collection of blood and tissue samples

Rats were decapitated while still under anaesthesia, and then trunk blood and tissue samples were collected. The PVN tissue was isolated following Palkovits's microdissection procedure as previously described (Kang et al., 2008; MohanKumar et al., 1998). Plasma and tissue samples were stored at −80 °C until assayed.