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Chronic infusion of lisinopril into hypothalamic paraventricular nucleus modulates cytokines and attenuates oxidative stress in rostral ventrolateral medulla in hypertension

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ABSTRACT

The hypothalamic paraventricular nucleus (PVN) and rostral ventrolateral medulla (RVLM) play a critical role in the generation and maintenance of sympathetic nerve activity. The renin–angiotensin system (RAS) in the brain is involved in the pathogenesis of hypertension. This study was designed to determine whether inhibition of the angiotensin-converting enzyme (ACE) in the PVN modulates cytokines and attenuates oxidative stress (ROS) in the RVLM, and decreases the blood pressure and sympathetic activity in renovascular hypertensive rats. Renovascular hypertension was induced in male Sprague–Dawley rats by the two-kidney one-clip (2K1C) method. Renovascular hypertensive rats received bilateral PVN infusion with ACE inhibitor lisinopril (LSP, 10 μg/h) or vehicle via osmotic minipump for 4 weeks. Mean arterial pressure (MAP), renal sympathetic nerve activity (RSNA), and plasma proinflammatory cytokines (PICs) were significantly increased in renovascular hypertensive rats. The renovascular hypertensive rats also had higher levels of ACE in the PVN, and lower level of interleukin-10 (IL-10) in the RVLM. In addition, the levels of PICs, the chemokine MCP-1, the subunit of NAD(P)H oxidase ($gp91^{phox}$) and ROS in the RVLM were increased in hypertensive rats. PVN treatment with LSP attenuated those changes occurring in renovascular hypertensive rats. Our findings suggest that the beneficial effects of ACE inhibition in the PVN in renovascular hypertension are partly due to modulation cytokines and attenuation oxidative stress in the RVLM.

Introduction

Both hypothalamic paraventricular nucleus (PVN) and rostral ventrolateral medulla (RVLM) play important roles in the regulation of sympathetic drive and cardiovascular activity. The number of PVN neurons projecting to the RVLM (PVN–RVLM neurons) is as much as sevenfold as the number of PVN neurons projecting to the spinal cord (Agarwal et al., 2011; Kumagai et al., 2012), and the PVN–RVLM pathway contributes to the change in sympathetic nerve activity (SNA) observed after activation of the PVN. The RVLM also contains presympathetic neurons that have spontaneous activity and directly project to the spinal cord (Kumagai et al., 2012). The basal activity of presympathetic neurons in the RVLM is a major mechanism responsible for the generation of resting blood pressure (BP) and sympathetic outflow (Kumagai et al., 2012; Zhang et al., 2008).

The renin–angiotensin system (RAS) plays an important role in the pathophysiology of cardiovascular disease (Veerasingham and Raizada, 2003). Angiotensin II (ANG II) is a principal and biologically active component of the RAS, exerts its actions mainly via interaction with the angiotensin II type 1 receptor (AT1-R), thereby contributing to sympathoexcitation and hypertensive response (Sriramula et al., 2011; Kasal and Schiffrin, 2012; Kumagai et al., 2012). AT1 receptors are widely distributed in the central nervous system from the forebrain to the brain stem (Xu et al., 1998). A growing body of evidence indicates that the main peptide of the RAS, ANG II, induces inflammatory molecules and contributes to the pathophysiology of cardiovascular diseases (Kang et al., 2009a,b; Qi et al., 2013). Recent studies also suggest that cytokines and RAS can contribute to these changes (Qi

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et al., 2013; Sriramula et al., 2013). Furthermore, data from our laboratory suggest that ANG II infusion increases proinflammatory cytokine (PIC) levels, such as tumor necrosis factor alpha-α (TNF-α), interleukin-1β (IL-1β) and interleukin-6 (IL-6) in plasma and PVN (Kang et al., 2009a,b), and ANG II blockade decreases PIC levels in plasma and PVN in cardiovascular disease (Kang et al., 2009a,b; Qi et al., 2013). It is well known that angiotensin-converting enzyme (ACE) promotes ANG II production through acting on the blood-born angiotensin I (ANG I). In addition, inhibition of ACE results in the reduction of PICs in the hearts of the spontaneously hypertensive rats (Miguel-Carrasco et al., 2010). However, whether inhibition of ACE in the PVN modulates cytokines in the RVLM and decreases the blood pressure and sympathetic activity during renovascular hypertension is still unclear.

Reactive oxygen species (ROS) in the RVLM is increased and contributes to the neural mechanisms of hypertension in stroke-prone spontaneously hypertensive rats (SHRSPs) (Kishi and Hirooka, 2012; Kishi et al., 2008) and spontaneously hypertensive rats (SHRs) (Koga et al., 2008). Our laboratory and others have shown that chronic peripheral ANG II infusion in rat induces hypertension, which is accompanied by superoxide accumulation in the RVLM or the PVN and increased sympathetic activity (Kang et al., 2009a,b; Zimmerman et al., 2004). Increasing evidence demonstrates that NAD(P)H oxidase derived ROS are important mediators of ANG II signaling (Iwanami et al., 2009). ANG II not only augments ROS formation, increases the oxidase activity, but also upregulates mRNA and protein expression of most NAD(P)H oxidase subunits in vitro and in vivo (Liu et al., 2008; Peng et al., 2013). In renovascular hypertensive rats, chronic oxidative stress within the RVLM is a major mechanism leading to chronic sympathoexcitation and high blood pressure (Oliveira-Sales et al., 2009).

Considering that the 2K1C model of arterial hypertension has a strong neurogenic component and central oxidative stress involved in the maintenance of high blood pressure, we tested the hypothesis that inhibition of ACE in the PVN attenuates 2K1C hypertensive responses and sympathoexcitation by the preferential reduction of oxidative stress and modulation of cytokines within the RVLM using lisinopril (LSP), an anti-hypertensive drug and potent reversible ACE inhibitor (Sharma and Singh, 2012).

Materials and methods

Animal. Experiments were performed on seven-week-old male Sprague–Dawley rats weighing between 275 g and 300 g. 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 (Kang et al., 2004; Yang et al., 1998). These experiments were approved by the Animal Care and Use Committee of Xi'an Jiaotong University and conformed to the Guidelines for the Care and Use of Experimental Animals of the United States National Institutes of Health (NIH Publication No. 85-23, revised 1996). All the following surgeries were performed under anesthesia and aseptic conditions.

General experimental protocol. After undergoing subcutaneously implantation of bilateral PVN cannulae, the rats were allowed a week for recovery. Measurement of baseline blood pressure (BP) was continuous for 3 days, and then renovascular hypertension was induced in male Sprague–Dawley rats for 4 weeks by the two-kidney one-clip (2K1C) method as previously reported (Han et al., 2011; Zhu et al., 2009). The osmotic minipumps (Alzet Model 1004, Durect Corporation, Cupertino, CA) were connected to the PVN cannulae for the continuous infusion of the ACE inhibitor lisinopril (LSP, 10 μg/h) or vehicle (artificial cerebrospinal fluid, aCSF) directly into the bilateral PVN over 4 weeks (Kang et al., 2009a,b). At the end of 4 weeks, 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 studies. Some rats were anesthetized for terminal electrophysiological studies.

Bilateral PVN cannulae implantation for chronic infusion studies. The method for implantation of bilateral PVN cannulae has been described previously (Qi et al., 2013). Briefly, after the rat was anesthetized with a ketamine (80 mg/kg) and xylazine (10 mg/kg) mixture (ip), the head was placed into a stereotaxic apparatus. The skull was then opened, and a stainless steel double cannula was implanted into the PVN according to 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. The histological identification was made to verify each injection site. The success rate of bilateral PVN cannulation is 64%, and only animals with verifiable bilateral PVN injection sites were used in the final analysis.

Mean arterial pressure (MAP) measurement. Blood pressure was determined by a tail—cuff occlusion and acute experiment method. Unanesthetized rats were warmed to an ambient temperature of 32 °C by placing rats in a holding device mounted on a thermostatically controlled warming plate. 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.

At the end of the 4th week, rats were anesthetized. The femoral artery was cannulated with polyethylene catheters for the measurement of arterial blood pressure (BP). The catheters were prior filled with 0.1 ml heparinized saline (50 units/ml) and connected to a pressure transducer attached to a digital BP monitor and a polygraph. MAP and heart rate (HR) data were collected for 30 min and averaged.

Electrophysiological recordings. Rats were anesthetized with a ketamine (80 mg/kg) and xylazine (10 mg/kg) mixture (ip).

A retroperitoneal incision was made and the left renal sympathetic nerve was isolated. The renal nerve was placed on a platinum electrode which is connected with the recording system and immersed in warm mineral oil. Maximum renal sympathetic nerve activity (RSNA) was detected using an intravenous bolus administration of sodium nitroprusside (SNP, 10 μg). At the end of the experiment, the background noise, defined as the signal-recorded postmortem, was subtracted from actual RSNA and subsequently expressed as percent of maximum (in response to SNP). The general methods for recording and data analysis have been described previously (Guggilam et al., 2007; Kang et al., 2008, 2010).

Collection of blood and tissue samples. Rats were decapitated under anesthesia with a ketamine (80 mg/kg) and xylazine (10 mg/kg) mixture (ip) to collect blood and tissue samples at the end of the 4th week of the experiment. Trunk blood samples were collected in chilled ethylenediaminetetraacetic acid tubes. Plasma samples were separated and stored at −80 °C until assayed for cytokines.

Tissue microdissection. Microdissection procedure was used to isolate the PVN and the RVLM as previously described (Gao et al., 2005). The tissues were collected from both sides of the PVN and the RVLM of individual rat.

Biochemical assays. The levels of IL-1β and IL-6 in plasma and tissue were quantified using commercially available rat ELISA kits (Biosource International Inc., Camarillo, California) according to the manufacturer's instructions. TNF-α in tissue was measured using a high sensitivity kit (GenStar Biosolutions Co., Beijing, China). According to the manufacturer's descriptions, the standards or sample diluents were added and incubated in the appropriate well of specific antibody precoated microtiter plate. Conjugate was added and incubated for 1 h at 37 °C and then washed. The reactions were stopped with stop