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Pro-inflammatory cytokines in paraventricular nucleus mediate the cardiac sympathetic afferent reflex in hypertension

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ABSTRACT

Our previous studies showed that pro-inflammatory cytokines (PIC) in the hypothalamic paraventricular nucleus (PVH) potentiated the cardiac sympathetic afferent reflex (CSAR) in normotensive rats. This study determined whether PIC in the PVH mediate enhanced CSAR and over-excited sympathetic activity in spontaneously hypertensive rats (SHR). CSAR was evaluated by renal sympathetic nerve activity (RSNA) response to epicardial application of bradykinin (BK). Inflammatory cytokine levels were measured with ELISA. In both SHR and normotensive Wistar-Kyoto (WKY) rats, PVH microinjection of PIC, tumour necrosis factor (TNF)-α or interleukin (IL)-1β, increased the baseline mean arterial blood pressure (MAP), RSNA and the CSAR, but anti-inflammatory cytokines (AIC), IL-4 or IL-13, only increased the baseline MAP. PVH pretreatment with PIC caused sub-response dose of angiotension (Ang) II to produce baseline RSNA and MAP elevation and the CSAR enhancement responses, but AIC (IL-4 or IL-13) did not. PVH microinjection of PIC induced greater changes in SHR than in normotensive WKY rats. In addition, stimulation of cardiac sympathetic afferents with epicardial application of BK increased PIC levels in the PVH in both SHR and WKY rats. Intrapericardial administration of resiniferatoxin (RTX) which abolished the CSAR decreased the PIC levels in the PVH to a lower level in SHR than in WKY rats. These results suggest that the increased PIC in the PVH in SHR mediated the increased sympathetic outflow and the enhanced CSAR, and that the augmented effect of Ang II in the PVH on sympathetic activity and the CSAR is also associated with PIC.

1. Introduction

It is well known that sympathetic activity is enhanced in hypertension (Ewen et al., 2014; Mancia et al., 1999; Schultz et al., 2007). This excessive sympathetic activation contributes to the pathogenesis and progression of hypertension. Intervention of the enhanced cardiac sympathetic afferent reflex (CSAR) to inhibit the over-excitation of sympathetic nervous system may be considered as a strategy for treating hypertension.

The hypothalamic paraventricular nucleus (PVH) is an integrative region that is important for the control of sympathetic outflow and arterial pressure through projections to the intermediolateral column of the spinal cord and the rostral ventrolateral medulla (RVLM) (Badoer, 2010; Coote, 2005). Some studies have shown that the PVH is an important central component in modulating the CSAR (Chen et al., 2011; Fan et al., 2012; Shi et al., 2011; Yuan et al., 2013; Zhong et al., 2008). On the other hand, some studies have shown that angiotensin (Ang) II and AT_1 receptors in the PVH play important roles in regulating CSAR and contribute to enhanced CSAR and sympathetic hyperactivity in heart failure and hypertension (Chen et al., 2011; Fan et al., 2012; Wang et al., 2005; Zhu et al., 2004).

It has been reported that pro-inflammatory cytokines (PIC) in the brain are novel molecules involved in the enhanced sympathetic nerve activity in rats with acute myocardial infarction (Francis et al., 2004a, 2004b), heart failure (Felder et al., 2003; Kang et al., 2008, 2010) and hypertension (Li et al., 2014; Su et al., 2014). We found that PIC, tumour necrosis factor (TNF)-α or interleukin (IL)-1β in the PVH, increase blood pressure and sympathetic outflow and enhance the CSAR in normal rats (Shi et al., 2011). There is a synergetic effect of Ang II with PIC on blood pressure, sympathetic activity and CSAR. The present study was designed to investigate whether inflammatory cytokines in the PVH are involved in the pathogenesis of enhanced CSAR in spontaneously hypertensive rats (SHR), in which the effects of PIC (TNF-α or IL-1β) and anti-inflammatory cytokines (AIC) (IL-4 or IL-13) were compared. Furthermore, synergetic effect of Ang II with TNF-α or IL-1β in the PVH on sympathetic activity and CSAR in SHR was determined.

Abbreviations: AIC, anti-inflammatory cytokines; Ang, angiotension; BK, bradykinin; CSAR, cardiac sympathetic afferent reflex; IL, interleukin; MAP, mean arterial blood pressure; PIC, pro-inflammatory cytokines; PVH, hypothalamic paraventricular nucleus; RTX, resiniferatoxin; RSNA, renal sympathetic nerve activity; SHR, spontaneously hypertensive rats; TNF, tumour necrosis factor; WKY, Wistar-Kyoto.

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2. Material and methods

2.1. Ethical approval

All animal work was approved and performed in accordance with the Home Office UK Animals (Scientific Procedures) Act 1986 under the regulations and policies laid out by the Medical Ethics Committee of Binzhou Medical University.

2.2. General procedures

Experiments were carried out in male SHR and normotensive Wistar-Kyoto (WKY) rats weighing between 300 and 350 g which were purchased from Vital River Laboratory Animal Technology Co. Ltd. (Beijing, China). Animals were housed on a 12-h light/dark cycle in a temperature-controlled room with standard chow and tap water ad libitum. Each rat was anaesthetized with intraperitoneal injection of urethane (800 $mg \cdot kg^{-1}$) and a-chloralose (40 $mg \cdot kg^{-1}$). Supplemental doses of anaesthesia were administered to maintain an adequate depth of anaesthesia during experiments. The rat was ventilated with room air using a rodent ventilator (683, Harvard Apparatus, South Natick, MA, USA). The right jugular vein and right carotid artery were cannulated for fluid infusion and recording of arterial blood pressure, respectively. At the end of the experiment, the rats were killed humanely by an overdose of pentobarbital sodium (100 $mg \cdot kg^{-1}$ intravenously).

2.3. Vagotomy and baroreceptor denervation

Vagotomy and baroreceptor denervation were carried out to minimize the secondary effect caused by baroreflex and cardiac vagal afferent reflex in rats and confirmed as previously reported (Han et al., 2005; Zhu et al., 2002). Bilateral carotid sinus nerves and vagi were identified and cut. The carotid bifurcation and the common carotid arteries were stripped of adventitial tissues from 4 mm below the bifurcation to 4 mm above. A 10% phenol solution was painted to vessels to destroy any remaining nerve fibres in carotid sinus fibres area.

2.4. Sympathetic nerve recordings

Renal sympathetic nerve activity (RSNA) was recorded as previously described (Han et al., 2007). The left renal sympathetic nerve was isolated through a retroperitoneal incision and cut distally and was placed on a pair of silver recording electrodes immersed in mineral oil. The signals of nerve were amplified with an AC/DC differential amplifier (Model 3000; A-M System, Washington, DC, USA) with a low-frequency cutoff at 60 Hz and a high-frequency cutoff at 3000 Hz. The amplified and filtered signals were integrated at time constant of 10 ms. Background noise was determined after section of the central end of the nerve and was subtracted from the integrated values of RSNA at the end of each experiment. The raw RSNA and the integrated RSNA were simultaneously recorded on a PowerLab data acquisition system (8SP; ADInstruments). After intervention, percentage change in integrated RSNA from the baseline value was calculated (Zhong et al., 2008).

2.5. Evaluation of CSAR

The heart was exposed and the pericardium was removed. The CSAR was elicited by stimulation of cardiac afferents with application of a piece of filter paper (3 × 3 mm) containing bradykinin (BK, 1.0 nmol in 2.0 μL) to the epicardial surface of anterior wall of the left ventricle as previously described (Han et al., 2007). Each piece of filter paper was removed 1 min later. Then 10 mL of warm normal saline (38 °C) was used to rinse the epicardium three times. The CSAR was evaluated by the RSNA and MAP responses to the epicardial application of BK.

2.6. Elimination of CSAR

In order to abolish the CSAR, intrapericardial administration of resiniferatoxin (RTX, 60 pmol) was used to desensitize transient receptor potential vanilloid 1 (TRPV1)-containing cardiac afferent fibres as previously reported (Zhu et al., 2009). To confirm the desensitizing effect of RTX on the cardiac afferent fibres, at the 120th minute after administration of RTX, the CSAR was determined by epicardial application of BK.

2.7. PVN microinjection

Rats were placed in stereotaxic instrument (Stoelting, Chicago, IL, USA). The coordinates for the bilateral PVH were determined according to the Paxinos and Watson rat atlas (1.8 mm caudal from the bregma, 0.4 mm lateral to the midline, and 7.9 mm ventral to the dorsal surface). The PVH microinjection volume in each side of the PVH was 50 nL and administration was completed within 1 min. When multiple injections in 1 animal are needed, the intervals between each microinjection were at least 40 min for a complete recovery. At the end of the experiment, 2% Evans blue dye (50 nL) was injected into the microinjection site. The microinjection site was verified histologically. Only data of the rats whose microinjection sites were within the boundaries of the PVH were used for data analysis. A representative photograph of microinjection sites in the PVH is shown in Fig. 1.

2.8. Measurements of TNF-α or IL-1β in the PVH

The brain of rats were removed after euthanized and quickly frozen with liquid nitrogen and stored at −70 °C until being sectioned. 450-μm-thick coronal slices were sectioned through the PVH levels. A 15-gauge needle (inner diameter 1.5 mm) was used to punch out the PVH area. Then the punched PVH was homogenized in ice cold saline and centrifuged. Protein concentrations in supernatant were measured with protein assay kit (BCA, Pierce, Rockford, IL, USA). TNF-α or IL-1β in the PVH was respectively measured using a rat TNF-α or IL-1β ELISA kit (R&D Systems; Oxfordshire, UK) according to the manufacturer's instructions. Briefly, a 96-well microplate was coated with an antibody specific for rat TNF-α or IL-1β. 100 μL of sample and 100 μL of standard diluent buffer were added to each well in duplicate, incubated it for 90 min at 37 °C. Then 100 μL of biotinylated anti-TNF-α or IL-1β antibody solution was added, incubated for 60 min at 37 °C. 100 μL of streptavidin horseradish peroxidase conjugate solution was added and incubated for 30 min at 37 °C. 100 μL of chromagen solution was added and incubated in the dark for 15 min at 37 °C. The reactions were stopped with HCl and results were read at 450 nm using an ELISA

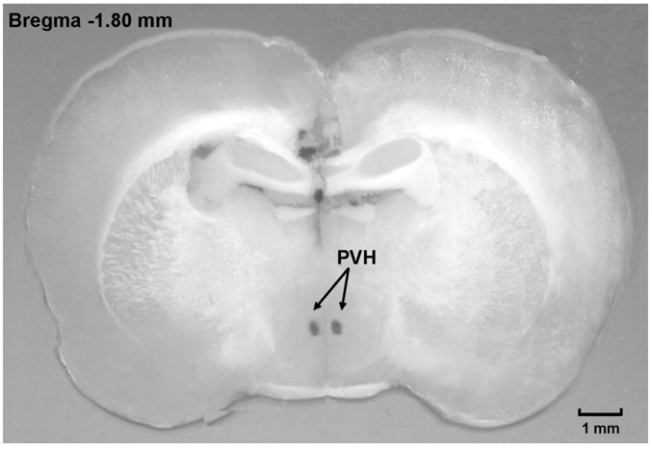

Fig. 1. A representative coronal section photograph showing bilateral PVH microinjection sites of a rat. The arrows show the microinjection sites.