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The asymmetric dimethylarginine-mediated inhibition of nitric oxide in the rostral ventrolateral medulla contributes to regulation of blood pressure in hypertensive rats

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ABSTRACT

Nitric oxide (NO) contributes to the central control of cardiovascular activity. The rostral ventrolateral medulla (RVLM) has been recognized as a pivotal region for maintaining basal blood pressure (BP) and sympathetic tone. It is reported that asymmetric dimethylarginine (ADMA), characterized as a cardiovascular risk marker, is an endogenous inhibitor of nitric oxide synthesis. The present was designed to determine the role of ADMA in the RVLM in the central control of BP in hypertensive rats. In Sprague Dawley (SD) rats, microinjection of ADMA into the RVLM dose-dependently increased BP, heart rate (HR), and renal sympathetic never activity (RSNA), but also reduced total NO production in the RVLM. In central angiotensin II (Ang II)-induced hypertensive rats and spontaneously hypertensive rat (SHR), the level of ADMA in the RVLM was increased and total NO production was decreased significantly, compared with SD rats treated vehicle infusion and WKY rats, respectively. These hypertensive rats also showed an increased protein level of protein arginine methyltransferases1 (PRMT1, which generates ADMA) and a decreased expression level of dimethylarginine dimethylaminohydrolases 1 (DDAH1, which degrades ADMA) in the RVLM. Furthermore, increased AMDA content and PRMT1 expression, and decreased levels of total NO production and DDAH1 expression in the RVLM in SHR were blunted by intracisternal infusion of the angiotensin II type 1 receptor (AT1R) blocker losartan. The current data indicate that the ADMA-mediated NO inhibition in the RVLM plays a critical role in involving in the central regulation of BP in hypertension, which may be associated with increased Ang II.

1. Introduction

Nitric oxide (NO) is well characterized for its physiological and pathological roles in cardiovascular regulation [1]. However, the NO role in neural regulation of blood pressure (BP) remains controversial in the rostral ventrolateral medulla (RVLM) which is a key region for maintaining basal BP and sympathetic tone [2]. The NO precursor L-arginine microinjected into the RVLM elicited hypotension, bradycardia, and reduction in sympathetic vasomotor tone in spontaneously hypertensive rat (SHR) [3]. Moreover, microinjection of NG-monomethyl-L-arginine (L-NMMA), a nitric oxide synthesis (NOS) inhibitor, into the RVLM induced a pressor response in hypertensive rats [4,5]. Overexpression of endothelial nitric oxide synthesis (eNOS) in the RVLM caused a greater sympathoinhibition in hypertensive rats than in normotensive WKY rat [6,7]. These results indicate that NO in the RVLM exerts sympathoinhibitory effect. On the other hand, overexpression of inducible NO synthesis (iNOS) in the RVLM increased BP in WKY rats and SHR, whereas bilateral microinjection of the iNOS selective inhibitor aminoguanidine into the RVLM reduced BP and heart rate (HR) in SHR [8]. In additional, it is suggested that NO in the RVLM exerts an excitatory effect on cardiac sympathoexcitatory responses [9]. Obviously, it is complex and variable that NO in the RVLM contributes to the pathogenesis of hypertension. Therefore, it is highly valuable to understand the potential mechanism of NO dysfunction in the RVLM for hypertensive subjects.

Asymmetric dimethylarginine (ADMA), an endogenous inhibitor of NOS, is generated by protein arginine methyltransferases

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(PRMT) and eliminated by dimethylarginine dimethylaminohydrolase (DDAH) [10]. Accumulating evidence has demonstrated that ADMA is characterized as a risk marker of cardiovascular disease events [11]. The elevated ADMA level in plasma has been observed in many cardiovascular diseases such as, atherosclerosis [12], coronary artery disease [13], stroke [14], and heart failure [15]. In peripheral, a previous study has reported that intravenous infusion of ADMA into healthy subjects increases systemic vascular resistance and elevates BP in a dose-related manner [16]. In additional, it has been demonstrated that ADMA plays a key role in the development and progression of salt-sensitive hypertension, associating with endothelial dysfunction *in vitro* [17] and *in vivo* [18]. However, the role of ADMA in mediating NO dysfunction in the RVLM and regulation of cardiovascular activities is not clear.

Overactivity of central renin-angiotensin system (RAS) plays a pivotal role in the pathogenesis of hypertension and related cardiovascular disorders [19]. It is reported that the pathological roles of angiotensin II (Ang II), a main factor of RAS, in neural mechanisms of hypertension are highly diverse. Likewise, multiple signaling pathways underlie the deleterious roles of Ang II in the RVLM in neurogenic hypertension. For example, Ang II not only enhances the level of oxidative stress in the RVLM via upregulation the NADPH oxidase [20], but also increases the level of inflammation via activation microglia [21]. On the other hand, a decrease in level of NO can lead to an increase in level of sympathetic nerve activity [22]. The balance between nitric oxide (NO) signaling and reactive oxygen species (ROS) in the RVLM is broken resulting in decreased baroreflex function, which contributes to neurogenic hypertension [23]. Interestingly, it has been reported that Ang II can increase the level of intracellular ADMA in the cultured endothelial cells [24]. However, whether ADMA in the RVLM is regulated by Ang II in hypertension is still an important goal in this study. Accordingly, two main aims in present study were designed to determine: 1) if ADMA in the RVLM has effects on central control of cardiovascular activities; 2) if the ADMA-mediated cardiovascular effect in the RVLM is associated with increased Ang II in hypertension.

2. Methods

2.1. Animals

Sixteen-week old male Sprague Dawley (SD) rats, WKY rats, and SHRs purchased from Sino-British SIPPR/BK Laboratory Animal Ltd (Shanghai, China) were used in this study. All procedures were obtained approval of the Institutional Animal Care and Use Committee of Second Military Medical University, and all operations in this study were conducted according to the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health.

2.2. General surgical procedures

Measurements of BP and HR *in vivo* were obtained as described in our previous study [25]. In brief, rats were anaesthetized by intraperitoneal injection of urethane (800 mg/kg) and a-chloralose (40 mg/kg). To facilitate mechanical respiration, the trachea was cannulated. The right femoral artery was catheterized for measuring BP and HR via a Powerlab system. The right femoral vein was cannulated for maintaining anaesthesia by supplementing α-chloralose (10 mg/kg). The body temperature was kept at 37 °C using a temperature controller.

2.3. Recording of RSNA

The raw RSNA was recorded and basal RSNA was further assessed, as previously described [26,27]. The left renal sympathetic nerve was exposed retroperitoneally; the discharge of renal sympathetic nerve was collected by a pair of recording electrodes. The signal of RSNA was amplified and recorded with the PowerLab system. Usually, the maximum nerve activity (Max) occurred 5 min after the rat was euthanized with an overdose of pentobarbital sodium (200 mg/kg). Background noise levels for sympathetic nerve activity were recorded 15–20 min after the rat was euthanized. Using the unit conversion of Powerlab Chart (AD Instruments) system, the Max was set to 100%, and the noise level was set to 0%. Baseline nerve activity was taken as percent of Max.

2.4. RVLM microinjection

The procedures of RVLM microinjections were based on our previous study [28]. In brief, the anaesthetized rat was fixed in a stereotaxic frame, and the dorsal surface of the medulla oblongata was exposed via resecting cervical muscles and occipital bone. Microinjections were made from a three-barrel micropipette. According to the atlas of rats [29], the microinjection site for RVLM was determined as follow: 2.0 mm lateral to the midline, 2.5 mm rostral to the obex, and 3.5 mm deep to dorsal surface of the medulla. Based on previous studies [22,30], the RVLM were functionally identified by a pressor response (>20 mmHg) evoked by microinjection of L-glutamate (1 nmol). The effects of microinjection of ADMA (0.01–2 nmol/100 nl, no.D4268, Sigma), L-arginine (10 nmol/100 nl, no.A5006, Sigma), L-NMMA (0.01–10 nmol/100 nl, no.M7033, Sigma) and AMI-1 (0.01–1 nmol/100 nl, No.S7884, Selleck) into the RVLM on BP, HR, and RSNA were observed. The artificial cerebrospinal fluid (aCSF) severed as vehicle control. At the end of experiment, the same microinjection sites were marked by 2% pontamine sky blue solution to confirm the injection sites located within the RVLM area. To detect the NO production in response to ADMA, the brain was removed at 15 min after microinjection of ADMA (1 nmol) into the RVLM in SD rats, and stored in −80 °C for Total NO production detection.

2.5. Intracisternal infusion of agents

The procedures of intracisternal infusion of agents were described in our previous study [31]. The rats were anaesthetized by inhaling 3% isofluorane and fixed in the stereotaxic frame. The atlantooccipital membrane was exposed after incising the cervical skin and muscles, a sterile needle punctured the dura, which confirmed entering the cisterna magna (fourth ventricle) by observing the leakage of cerebrospinal fluid from the hole. Next, a PE-10 catheter was inserted into the fourth ventricle for 3 mm, fixed the catheter and sealed to the dura with tissue glue. The outer end of the catheter connected to an osmotic mini-pump (Model 1007D or 1002, Alzet, USA) containing Ang II or the angiotensin II type 1 receptor (AT1R) blocker losartan. The doses of intracisternal infusion of agents (Ang II, 20 μg/kg/day, one week; losartan 1 mg/kg/day, two weeks, Sigma) were based on previous studies [32,33]. After completion of intracisternal infusion, the rats were euthanized by overdose of pentobarbital sodium (200 mg/kg), thus the brain was removed and stored in −80 °C.

2.6. Western blot analysis

The protocols of Western blot were described previously [22]. The RVLM tissue was punched according to the rat brain atlas [29], lysed, sonicated, and centrifuged. The protein samples were