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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

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collected by extracting the supernatants, the protein concentration of every sample was measured using BCA kit. The protein samples were denatured with loading buffer in proportion heating to 100 °C for 10 min and loaded onto a 10% SDS–PAGE gel and then transferred to PVDF membrane. The membrane was incubated with primary antibody [anti-PRMT1 (no.ab190892, abcam); anti-DDAH1 (no.ab180599, abcam); and anti-DDAH2 (no.ab184166, abcam)] after blocking for 2 h with 5% milk dissolved in Tris-buffered saline Tween overnight at 4 °C, After 3 times washes, secondary antibodies conjugated horseradish peroxidase were used to interact with primary antibodies for 2 h in room temperature. The protein bands were visually detected with chemiluminescent agent and analyzed by GeneTools software (Gene Company). The expression level of target proteins was normalized to α-tubulin.

2.7. Elisa assay

The procedure of protein extraction from RVLM tissues for Elisa assay was consistent with protein sample preparation for Western blot experiment. The supernatants were extracted after centrifugation, the protein concentration of every protein sample was measured using BCA kit, and the level of ADMA was determined by Elisa kits (Shanghai Westang Bio-tech Co., LTD) according to the manufacturer's instructions. The level of ADMA were determined the absorbance at 450 nm with an automated micro plate reader, and were calculated for each sample according to the standard curve.

2.8. Total NO production detection

According to previous study [34], the NO production in RVLM was detected. The RVLM tissue was punched and lysed with cell and tissue lysis buffer (purchased from Beyotime Biotechnology, #S3090) special for Nitric Oxide Assay, sonicated, and centrifuged. The supernatants were extracted after centrifugation; the protein concentration of each sample was measured using BCA kit. Total NO production in the RVLM tissue was determined by measuring the concentration of nitrate and nitrite, a stable metabolite of NO, using the Total Nitric Oxide Assay Kit (purchased from Beyotime Biotechnology, #S0023), according to the manufacturer's instructions. In brief, 60 μl of each extracted RVLM sample was added in the 96 well plates, each sample was incubated with 5 μl of nicotinamide adenine dinucleotide phosphate (NADPH) (2 mM), 10 μl of flavin adenine dinucleotide (FAD), and 5 μl of Nitrate Reductase at 37 °C for 30 min; then, 10 μl of lactate dehydrogenase (LDH) Buffer and 10 μl of LDH were added in the above mixed reaction buffer, incubating at 37 °C for 30 min again. Finally, 50 μl of Griess Reagent I and 50 μl of Griess Reagent II were added into all reaction wells. After mixing, the 96 well plates were placed at room temperature for 30 min. The level of nitrate and nitrite were determined by the absorbance at 540 nm with an automated micro plate reader, and were calculated for each sample according to the standard curve.

2.9. Data analysis

All values are expressed as mean ± SE. BP is presented by mean arterial pressure. Student's *t*-test (unpaired) was used for comparing the differences in cardiovascular parameters, ADMA content, total NO production, and the expression levels of PRMT1, DDAH1, and DDAH2 protein between SD rats treated with aCSF and Ang II. Above parameters in differences between WKY and SHRs treated with or without losartan, as well as the changes in cardiovascular activities after microinjection of different doses of ADMA and L-NMMA into the RVLM were analyzed by the one-way ANOVA followed by Bonferroni's *post hoc* test. The differences in percent changes of BP, HR, and RSNA before and after microinjection of ADMA or AMI-1 into the RVLM were also analyzed by Student's t-test. Differences were considered to be significant at $P < 0.05$.

3. Results

3.1. Effects of microinjection of ADMA into the RVLM on cardiovascular activity and total NO production in the RVLM

There are no differences in basal BP and HR before microinjection of ADMA into the RVLM among several groups (Table 1). As shown in Fig. 1, escalating doses of ADMA range from 0.01 nmol to 2 nmol were bilaterally microinjected into the RVLM in SD rats. It was found that 0.01 nmol of ADMA microinjection did not significantly change BP, HR, and RSNA (BP: 2.1 ± 1.0 mmHg; HR: 3.8 ± 1.5 bpm; RSNA: 3.9 ± 0.7%), compared with microinjection of aCSF (BP: 0.9 ± 1.3 mmHg; HR: 0.3 ± 1.7 bpm; RSNA: 0.4 ± 1.0%). The other doses of microinjection of ADMA into the RVLM produced a significant dose-dependent increase in BP. It was confirmed that the cardiovascular effects of 1 nmol ADMA microinjection were most obvious and lasted for at least 15 min, and maximum effects of 1 nmol ADMA in cardiovascular activities are not less than the effects of 2 nmol ADMA. Therefore, 1 nmol ADMA as optimal dose was selected in the following experiments. Furthermore, total NO production was significantly decreased in the RVLM after microinjection of ADMA (1 nmol) into the RVLM compared with microinjection of aCSF (2.633 ± 0.818 vs. 5.380 ± 0.479 nmol/mg protein) (Fig. 1C). Furthermore, compared with aCSF, microinjection of L-arginine (10 nmol) into the RVLM significantly decreased BP (1.0 ± 1.8 vs. −16.2 ± 4.0 mmHg), but also prevented the ADMA-induced increase in BP, HR and RSNA (BP: 18.2 ± 1.9 vs. 6.8 ± 1.6%; HR: 10.6 ± 3.0 vs. −0.8 ± 0.9%; RSNA: 33.9 ± 6.8 vs. 8.7 ± 1.2%) (Fig. 2). We also detected the effects of microinjection of monomethyl-L-arginine (L-NMMA, a precursor of ADMA) into the RVLM on cardiovascular activity. The levels of baseline BP and HR in several groups for L-NMMA injections were shown in Table 2. As indicated in Fig. 3, it was found that bilateral microinjections of L-NMMA (0.01–10 nmol) into the RVLM produced a dose-dependent increase in BP, HR, and RSNA.

3.2. Effects of intracisternal infusion of Ang II on ADMA content and total NO production in the RVLM

As indicated in Fig. 4, central infusion of Ang II (20 μg/kg/day, 1 week) significantly increased cardiovascular activities (Table 3), ADMA content (0.353 ± 0.031 vs. 0.268 ± 0.009 nmol/mg protein) and decreased total NO production (2.934 ± 0.430 vs. 5.170 ± 0.638 nmol/mg protein) in the RVLM compared with intracisternal infusion of aCSF. As indicated in Fig. 5, we observed that Ang II infusion induced a significant increase in protein expression of PRMT1 and decrease in protein expression level of

Table 1
Baseline values of BP and HR before microinjection of escalating doses of ADMA into the RVLM in SD rats.

	BP(mmHg)	HR(bpm)
aCSF	106 ± 4	379 ± 15
0.01 nmol ADMA	112 ± 4	382 ± 10
0.1 nmol ADMA	104 ± 7	370 ± 19
1 nmol ADMA	103 ± 2	366 ± 16
2 nmol ADMA	108 ± 5	380 ± 13

Data are presented as mean ± SEM, bpm, beats per minute. Five rats in each group.

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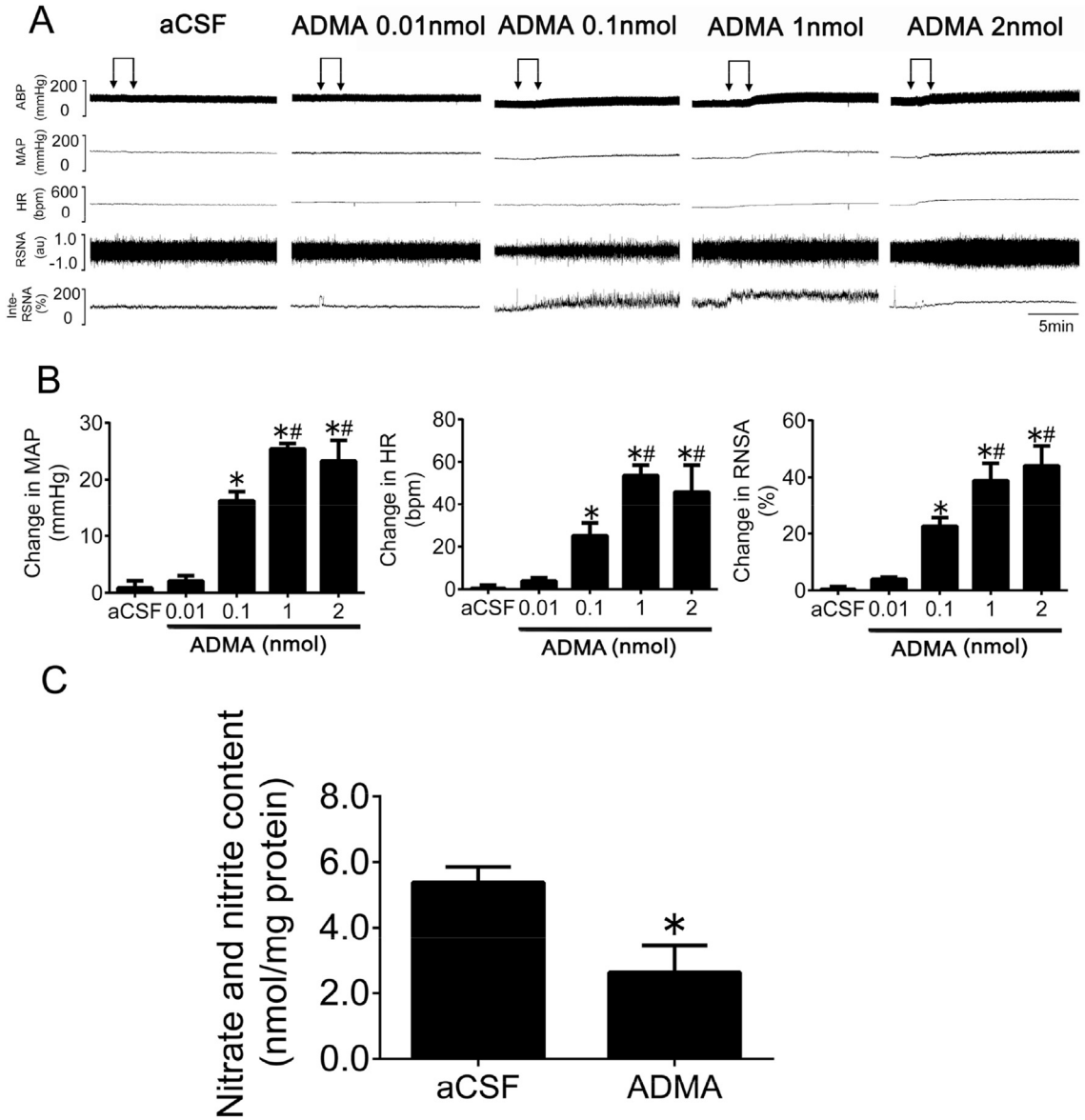

Fig. 1. Effects of microinjection of ADMA into the RVLM on cardiovascular activity (BP, HR, and RSNA) and total NO production in the RVLM in SD rats. The representative original tracings (A) and peak changes (B) of BP, HR, and RSNA in response to microinjections of aCSF and escalating doses of ADMA (0.01 nmol−2 nmol) into the RVLM. (C) Total NO production in the RVLM after microinjection of ADMA (1 nmol) into the RVLM. Data are presented as mean ± SEM, n = 5/group, *P < 0.05 vs. aCSF; #P < 0.05 vs. 0.1 nmol group ADMA.

DDHA1. However, there was no significant difference in the expression of DDAH2. Moreover, bilateral microinjection of AMI-1 (0.01−1 nmol) into the RVLM has little effect on resting BP and RSNA in normal SD rats. In the Ang II-induced hypertensive rats, however, microinjection of AMI-1 (1 nmol) into the RVLM produced a significant decrease in BP and RSNA (Fig. 6).

3.3. Effects of central infusion of losartan on ADMA content and total NO production in the RVLM in SHR

As indicated in Fig. 7, the level of ADMA (0.354 ± 0.012 vs. 0.244 ± 0.007 nmol/mg protein) is increased and total NO production (3.075 ± 0.282 vs. 5.728 ± 0.508 nmol/mg protein) decreased significantly in the RVLM in SHR compared with WKY rats. SHR also showed a significant increase of 41% in protein expression level of PRMT1 and decrease of 65% in protein expression level of DDAH1 (Fig. 8). Moreover, these alterations in SHR were significantly blunted by central infusion of the AT1R blocker losartan (1 mg/kg/day, 2 weeks). In additional, there is no significant difference of the DDAH2 expression among these experimental groups.

4. Discussion

In present study, three major findings are that: 1) Microinjection of ADMA into the RVLM evokes a significant increase in BP, HR, and RSNA, as well as a significant decrease in total NO production in the RVLM. Moreover, the pressor response to ADMA was significantly blunted by pretreatment with L-arginine; 2) In the central Ang II-induced hypertensive rats and SHR, the ADMA content is increased and total NO production is decreased in the RVLM, which may be associated with up-regulation of PRMT1 and down-regulation of DDAH1; and 3) These alterations in SHR were blunted by central infusion of losartan. Based on the above findings, it is

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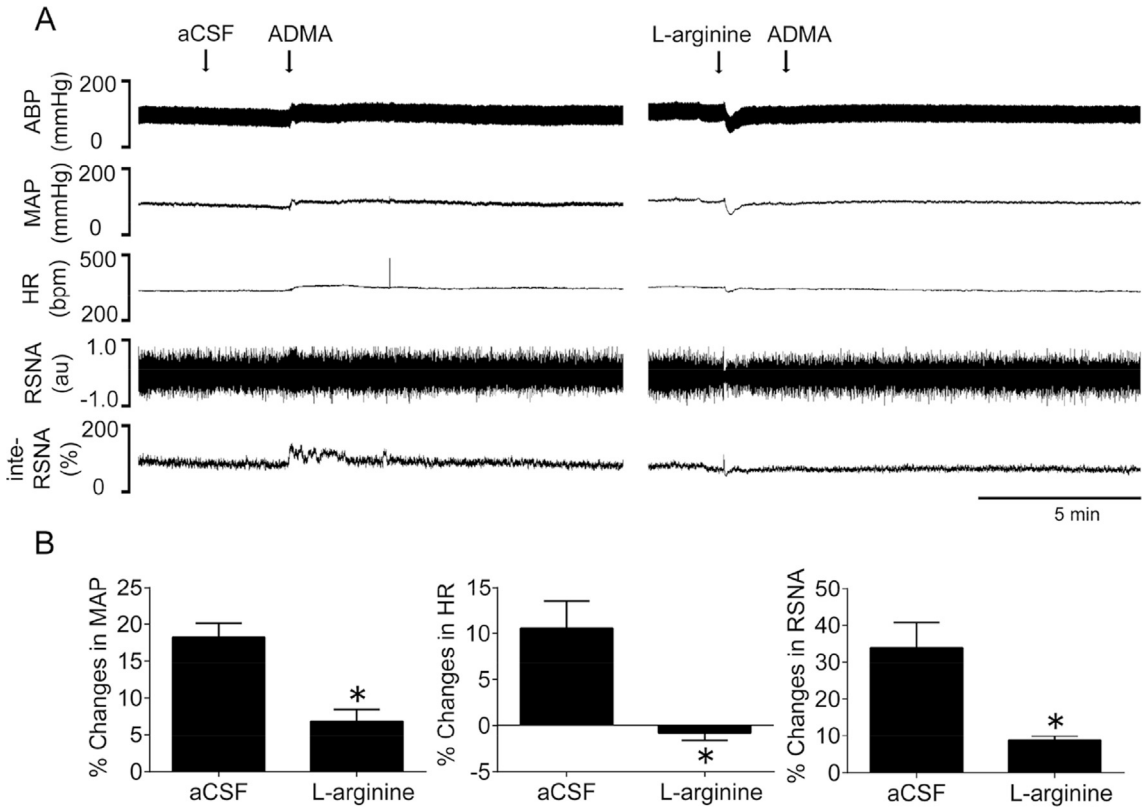

Fig. 2. Effects of microinjection of L-arginine into the RVLM on the ADMA −induced cardiovascular effects in SD rats. The representative original tracings (A) and the percent peak changes (B) of BP, HR, and RSNA in response to microinjection of ADMA (1 nmol) into the RVLM after pretreatment with aCSF and L-arginine (10 nmol). Data are presented as mean ± SEM, n = 5/group, *P < 0.05 vs. aCSF.

Table 2
Baseline values of BP and HR before microinjection of escalating doses of L-NMMA into the RVLM in SD rats.

	BP(mmHg)	HR(bpm)
aCSF	110 ± 3	359 ± 14
0.01 nmol l-NMMA	109 ± 3	372 ± 11
0.1 nmol L-NMMA	114 ± 6	375 ± 9
1 nmol L-NMMA	108 ± 7	363 ± 13
10 nmol L-NMMA	107 ± 4	373 ± 18

Data are presented as mean ± SEM, bpm, beats per minute. Five rats in each group.

indicated that ADMA in the RVLM plays a critical role in regulation of BP in hypertension, which is associated with increased Ang II.

In our study, we observed an excitatory effect induced by ADMA microinjected directly into the RVLM on cardiovascular activities. Moreover, microinjection of L-NMMA into the RVLM also produced an increase in BP, HR, and RSNA. These results suggested that MMA could also exert cardiovascular regulatory effects in the RVLM. Three are three types of arginine methylation in mammalian cells: MMA, ADMA and symmetric dimethylarginine (SDMA). PRMT1 is the primary methyltransferase that deposits the ADMA mark, and it accounts for over 90% of this type of methylation. Moreover, the loss of PRMT1 function mainly induces an increase in global MMA and SDMA levels. Amino acid analysis confirms that MMA and SDMA levels accumulate when ADMA levels are reduced [35]. These findings show the dynamic interaction between three arginine methylation types in the cells. Therefore, we suppose that the content of MMA in the RVLM in hypertensive rats probably be decreased, due to up-regulation of the protein expression of PRMT1 and increase in the level of ADMA.

Abundant amounts of documents mainly focus on the role ADMA in endothelial dysfunction, and have demonstrated that oxidative stress, eNOS inhibition, eNOS uncoupling, inflammation, and shear stress play an important role in ADMA pathophysiology in vascular endothelial cell by regulating NO synthesis [36]. In additional, NO deficiency in the RLVM is involved in neurogenic hypertension and baroreflex impairment [37,38]. In this work, microinjection of ADMA into the RVLM produced a significant decrease in total NO production. Moreover, the pressor response of ADMA was significantly inhibited by pretreatment with L-arginine, suggesting a possibility that cardiovascular effect of ADMA is associated with NOS inhibition in the RVLM. There are four members of the family: neuronal NOS (nNOS), eNOS, iNOS, and mitochondrial NOS (mtNOS), and all of which are expressed in the brain [23]. However, all isoforms of NOS, rather than mtNOS, are present in the RVLM [39] and have a discrepant effect on sympathetic outflow in the RVLM. For example, upregulation the eNOS/NO signaling with adenovirus vectors encoding eNOS in the RVLM exerts sympathoinhibition under hypertensive conditions [7]. In heart failure rats, ablation in nNOS expression and NO production in the RVLM results in the elevated sympathetic outflow, whereas overexpression of nNOS and increase NO level in the RVLM attenuates the sympathetic overdrive [40]. The iNOS expression in the RVLM of hypertensive rats is inconsistent (upregulation [41] or downregulation [42] of iNOS) in different studies. However, overexpression of iNOS in the RVLM increased BP in WKY rats and SHR, whereas bilateral microinjection of iNOS selective inhibitor aminoguanidine into the RVLM dose-dependently reduced BP and HR in SHR [8]. Although ADMA is NOS inhibitor for all NOS isoforms [43], we could deduce that ADMA mainly inhibited the activity of eNOS and nNOS, rather than iNOS, according to the excitatory

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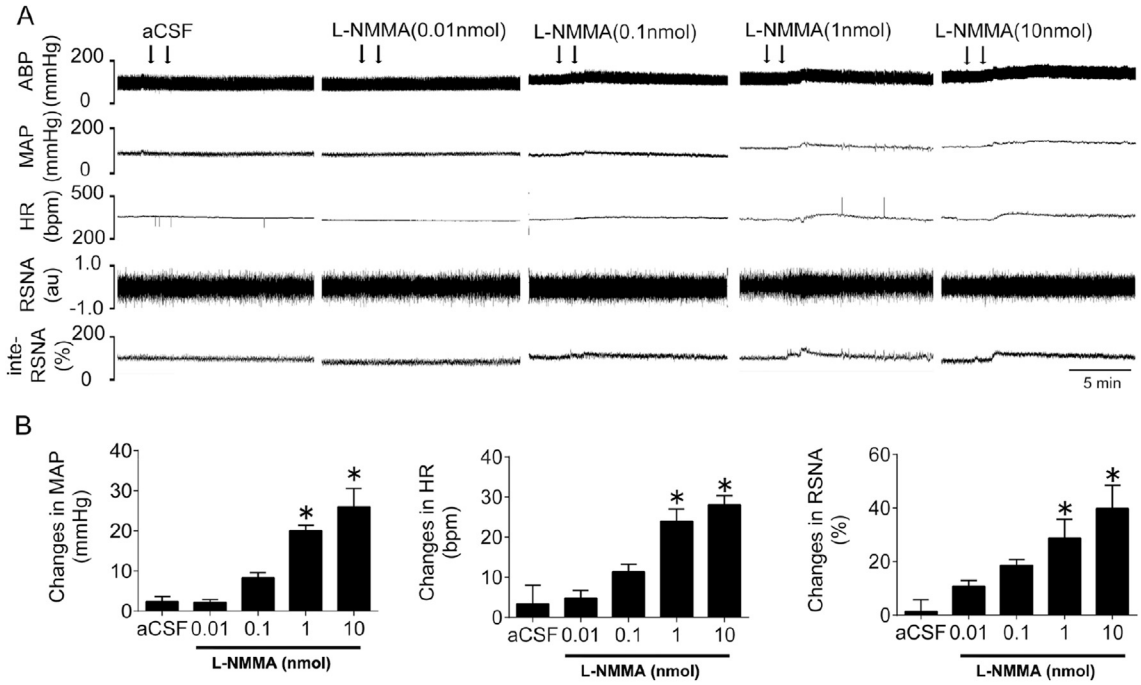

Fig. 3. Effects of microinjection of L-NMMA into the RVLM on cardiovascular activity in SD rats. The representative original tracings (A) and maximum changes (B) of BP, HR, and RSNA in response to microinjections of aCSF and escalating doses of L-NMMA (0.01 nmol−10 nmol) into the RVLM. Data are presented as mean ± SEM, n = 5/group, *P < 0.05 vs. aCSF.

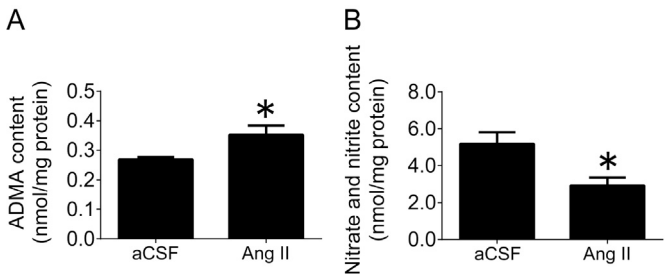

Fig. 4. Effects of intracisternal infusion of Ang II (20 μg/kg/day, one week) on ADMA content (A) and total NO production (B) in the RVLM in SD rats. Data are presented as mean ± SEM, n = 5/group, *P < 0.05 vs. aCSF.

Table 3
Effects of intracisternal infusion of Ang II and losartan on BP, HR and RSNA in the anaesthetized rats.

	BP (mmHg)	HR (bpm)	RSNA (%)
SD + aCSF	116 ± 3	375 ± 13	10 ± 1
SD + Ang II	146 ± 4*	432 ± 12*	23 ± 2*
WKY	120 ± 1	365 ± 15	16 ± 1
WKY + Los	117 ± 4	345 ± 8	13 ± 1
SHR	179 ± 4§	413 ± 8§	38 ± 1§
SHR + Los	147 ± 2#	359 ± 12#	24 ± 2#

Data are presented as mean ± SEM, n = 5. Los, losartan. bpm, beats per minute. Five rats in each group. *P < 0.05 vs. aCSF. §P < 0.05 vs. WKY; #P < 0.05 vs. SHR.

cardiovascular effect and decreased total NO production caused by ADMA in this study. Clearly, this question needs to be defined in future.

In this study, it is observed that central infusion exogenous Ang II also increases ADMA content in the RVLM, accompanied with decreased NO, and elevated BP, HR, and RSNA. In SHR, central blockade of the AT1 receptor by losartan significantly decreased ADMA content and normalized NO production in the RVLM. ADMA was derived from methylation of arginine residues by protein arginine methyltransferases, which including nine subtypes from PRMT1 to PRMT9 [10], and PRMT1 is the main enzyme for catalyzing ADMA generation [44]. ADMA elimination is mediated mainly by degradation via dimethylarginine dimethylaminohydrolase, which including DDAH1 and DDAH2 [45]. However, there was a limitation that we did not further provide the related signaling pathway involved in the upregulation of PRMT1 induced by Ang II in this study. The possible signaling pathway involved in the upregulation of PRMT1 expression caused by Ang II needs further experiment to determine. However, the related signaling pathway in regulation of DDAH has been suggested. For example, blocking AT1R and protein expression of DDAH via activation of peroxisome proliferator activated receptor gamma (PPAR γ) signaling [46]. In additional, anti-inflammatory cytokine interleukin-10 (IL-10) prevents the Ang II-induced hypertensive effects in vascular smooth muscle cells of SHR via increasing protein expression and activity of DDAH1 [47]. In fact, there are many underlying mechanisms involving in the inhibitory effects of Ang II on NOS expression and activity. For example, Ang II limits NO production by upregulating arginase through a p38 MAPK-ATF-2 pathway in bovine aortic endothelial cells [48]. It is also reported that Ang II impairs the NOS activity by overstimulating NADPH oxidase in SHR [49]. Although the present data suggest that upregulation of PRMT1 and ADMA is a potential mechanism for the regulation of NOS activity by Ang II, we do not exclude the other Ang II-mediated mechanisms involved in impairment of NOS activity. In additional, many studies have demonstrated that nNOS expression in the RVLM remains unchanged [50,51] or upregulated [52] in SHR. in this work, our results showed that the NOS activity was decreased in the RVLM in SHR, suggesting that the decreased NOS activity may be not resulted from the change in NOS expression. Therefore, the inhibitory factors of NOS activity may play important role in decrease in NOS activity in hypertensive rats.

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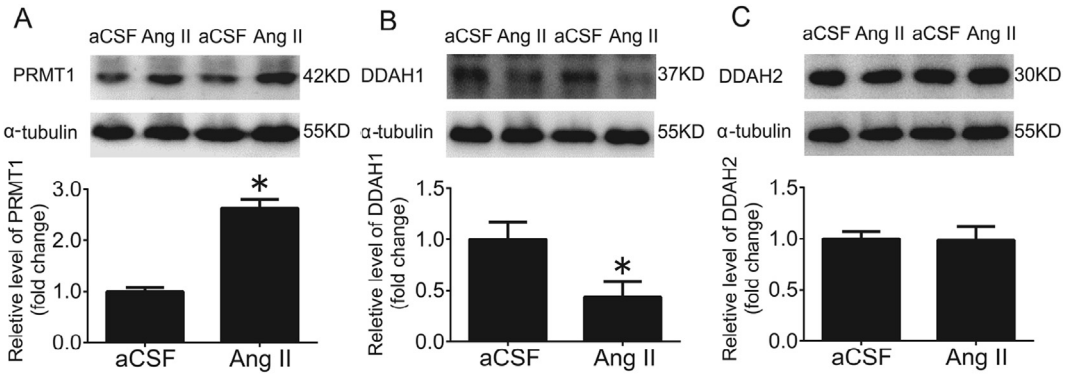

Fig. 5. Effects of intracisternal infusion of Ang II on PRMT1, DDAH1, and DDAH2 expression in the RVLM in SD rats. Representative gel bands (top) and quantification histogram (bottom) of PRMT1 (A), DDAH1 (B), and DDAH2 (C) expressions in the RVLM in SD rats after pretreatment with aCSF or Ang II (20 μg/kg/day, one week). Data are presented as mean ± SEM, n = 5/group, *P < 0.05 vs. aCSF.

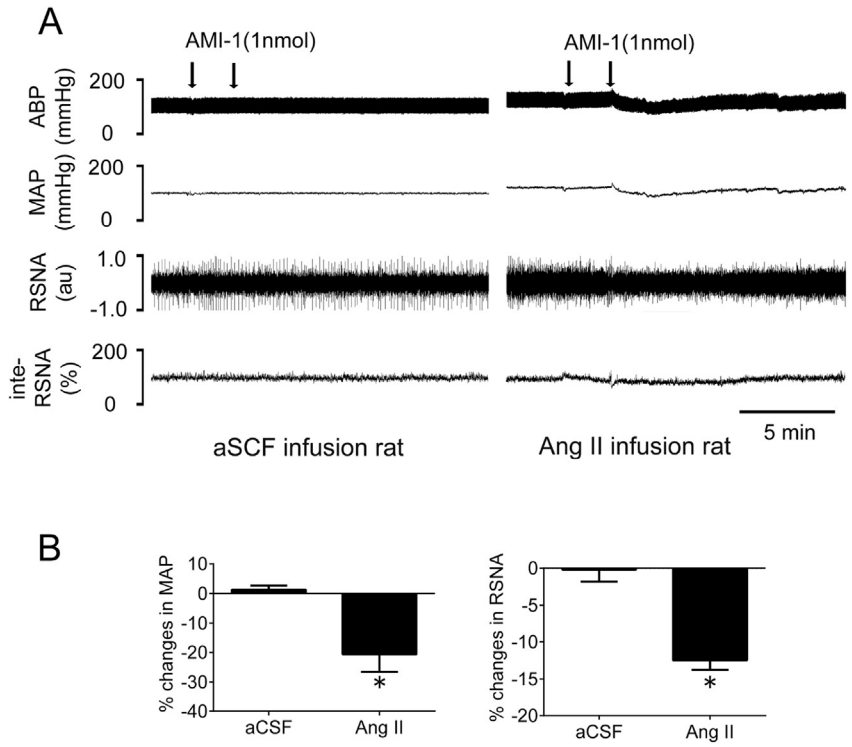

Fig. 6. Effects of microinjection of AMI-1 into the RVLM on cardiovascular activity in the Ang II-induced hypertensive rats. The representative original tracings (A) and the percent peak changes (B) of BP and RSNA in response to microinjection of AMI-1 (1 nmol) into the RVLM in rats after pretreatment with intracisternal infusion of aCSF and Ang II (20 μg/kg/day, one week). Data are presented as mean ± SEM, n = 5/group, *P < 0.05 vs. aCSF.

Importantly, our results further demonstrated that the NOS inhibitor ADMA was increased in the RVLM in hypertensive rats. Blockade of ADMA production by AMI-1 pretreatment in the RVLM produced a significant decrease in BP and RSNA in the Ang II-induced hypertensive rats. These data indicate that the PRMT1-ADMA-NOS axis in the RVLM plays an important role in the pathogenesis of chronic hypertensive state.

In this study, it may be a limitation that the potential role of ADMA/MMA on oxidative stress in the RVLM is not further determined. Previous evidence suggests that the interaction between ADMA and oxidative stress is an important mechanism involved in the pathogenesis of hypertension [53]. For example, increase in reactive oxygen species (ROS) in young rats with bile-duct-ligation model and *in vitro* cultured hepatocytes leads to downregulation of DDAH 1 and DDAH 2 as well as DDAH activity and upregulation of PRMT, leading to an increase in ADMA [53–55]. On the other hand, the NOS uncoupling produces peroxynitrite in the presence of high ADMA levels in Bovine aortic endothelial cells, which leads to increase the level of ROS [56]. Thus, ADMA may contribute to the production of ROS and reactive nitrogen species. It has been documented that increased ROS in the RVLM contributes to sympathetic overactivity and high BP in rats with hypertension and chronic heart failure [57–59]. Therefore, confirmation that oxidative stress is involved in the RVLM ADMA-mediated cardiovascular effect is helpful to our understanding the pathogenesis of hypertension.

In summary, ADMA is confirmed to contribute to central regulation of BP and sympathetic outflow, which might be associated

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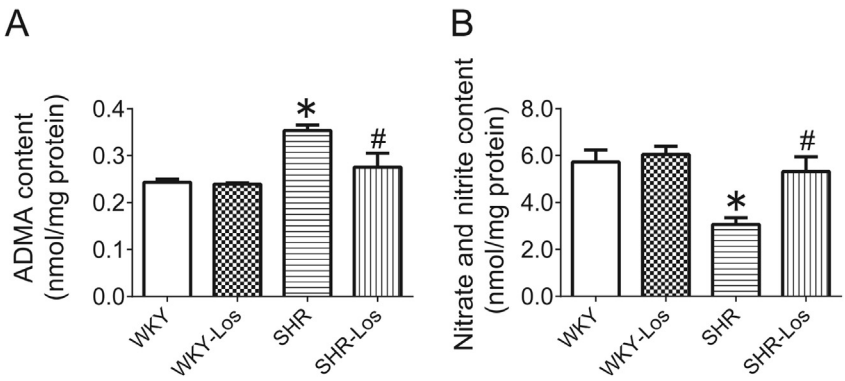

Fig. 7. Effects of intracisternal infusion of losartan (1 mg/kg/day, 2 weeks) on ADMA content (A) and total NO production (B) in the RVLM in WKY rats and SHR. Los: losartan. Data are presented as mean ± SEM. n = 5/group, *P < 0.05 vs. WKY; #P < 0.05 vs. SHR.

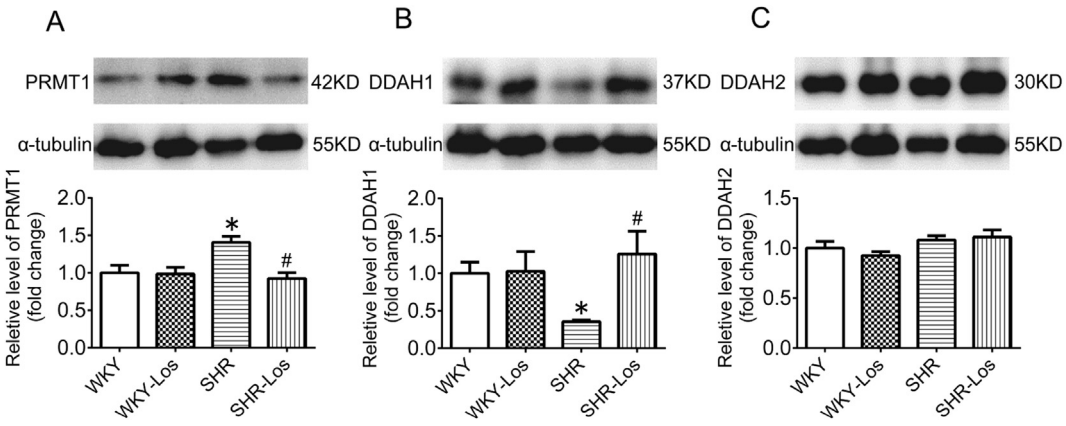

Fig. 8. Effects of intracisternal infusion of losartan on PRMT1, DDAH1, and DDAH2 expression in the RVLM in WKY rats and SHR. Representative gel bands (top) and quantification histogram (bottom) for PRMT1 (A), DDAH1 (B), and DDAH2 (C) expression in the RVLM in WKY rats and SHR after pretreatment with or without central losartan (1 mg/kg/day, 2 weeks). Data are shown as mean ± SEM, n = 5/group, *P < 0.05 vs. WKY; #P < 0.05 vs. SHR.

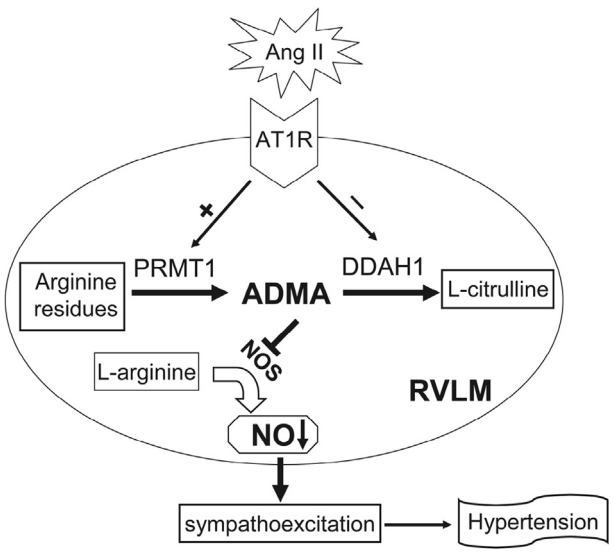

Fig. 9. The schematic of the relationship between Ang II and ADMA involved in central control of BP in hypertension. +, upregulation; -, downregulation.

with NO reduction. Moreover, Ang II increased ADMA in the RVLM via upregulation of PRMT1 and downregulation of DDAH1, which inhibited the NOS activity and decreased total NO production and resulted in high level of sympathetic tone and BP (Fig. 9). This work provides new evidence to our understanding of the ADMA-mediated NO release involved in central regulation of BP in hypertension. The increased ADMA along with NO inhibition in the RVLM caused by Ang II plays an important role in the pathogenesis of hypertension. Strategies that decrease ADMA in the central nervous system may be a possible therapeutic targeting for hypertension.

Disclosures

No conflicts of interest.

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Abbreviations and acronyms

aCSF	Artificial cerebrospinal fluid
ADMA	asymmetric dimethylarginine
Ang II	angiotensin II
AT1R	angiotensin II type 1 receptor
BP	blood pressure
CNS	central nervous system
DDAH1	dimethylarginine dimethylaminohydrolases 1
DDAH2	dimethylarginine dimethylaminohydrolases 2
eNOS	endothelial NOS
HR	heart rate
iNOS	inducible NOS
L-NMMA	NG-monomethyl-L-arginine
mtNOS	mitochondrial NOS
nNOS	neuronal NOS
NO	nitric oxide
NOS	nitric oxide synthesis
PRMT1	protein arginine methyltransferases1
PVN	hypothalamic paraventricular nucleus
RAS	renin-angiotensin system
ROS	reactive oxygen species
RSNA	renal sympathetic nervous activity
RVLM	rostral ventrolateral medulla
SD rats	Sprague Dawley rats
SHR	spontaneously hypertensive rat
WKY rats	Wistar-Kyoto rats

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