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Downregulation of cystathionine γ lyase and endothelial nitric oxide synthase and reduced responsiveness of α_{1A} adrenergic receptors in the kidneys of left ventricular hypertrophied Wistar Kyoto rats

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Abstract: This article explores the relationship between the renal expression of cystathionine gamma lyase (CSE) and endothelial nitric oxide synthase (eNOS) and the responsiveness of α_{1A} adrenergic receptors in the renal vasculature following left ventricular hypertrophy (LVH) in rats. LVH was established by administering isoprenaline (5 mg/kg, 5 injections subcutaneously, 72 h apart) with 62 mg/L caffeine in drinking water for 2 weeks. Renal vasoconstrictor responses were measured using local administration of adrenergic agonists noradrenaline (NA), phenylephrine (PE), and methoxamine (ME) and the selective α1A adrenergic antagonist 5-methylurapidil (5-MeU). Mean arterial blood pressure was higher (144 ± 9 vs. 116 ± 4 mmHg) and renal cortical blood perfusion was lower in the LVH group (102 ± 5 vs. 157 ± 19 bpu) compared to the control group ($P < 0.05$). There was a 68% downregulation of mRNA for renal CSE and 79% for eNOS in the LVH group compared to the control group (taken as 100%) ($P < 0.05$). The high dose of 5-MeU attenuated the vasoconstrictor responses to NA by 33%, PE by 44%, and ME by 43% in the LVH group compared to the same dose in the control group. The reductions in basal renal cortical perfusion and α_{1A} adrenergic receptor vasoconstrictor responses in LVH were associated with the downregulation of the CSE/H2S and eNOS/NO pathways.

Key words: Adrenergic receptors, isoprenaline, caffeine, left ventricular hypertrophy

1. Introduction

The development of left ventricular hypertrophy (LVH) occurs because of many factors, including increased heart work and the sympathetic nervous system. Activation of the sympathetic nervous system is associated with an increase in left ventricle mass (Burns et al., 2007) and may occur because of increased workload on the heart due to a raised total peripheral resistance. It has been observed that the addition of adrenergic agonists to perfusing media stimulates the in vitro growth of myocytes and the replication of vascular smooth muscle cells, whereas both phenomena can be blocked by adding adrenergic antagonists (Sen et al., 1974). The isoprenaline and caffeine model (I/C model) of LVH is known to result in elevated plasma levels of noradrenaline (NA) and angiotensin II (Daly et al., 1971; Leenen et al., 2001). Increased sympathetic nerve activity is known to have an impact on vascular function and impairs α_1 adrenoceptor-mediated renal vasoconstriction (Sun and Hanig, 1983). The functional contribution of α_1 adrenergic receptors in different disease states with sympathetic hyperactivity has been studied (Sattar and Johns, 1994a, 1994b). However, little information is available as to the impact of LVH on the responsiveness of the renal vasculature to exogenously administered adrenergic agonists.

Hydrogen sulfide (H_2S), carbon monoxide (CO), and nitric oxide (NO) are endogenously produced lipid-soluble gaseous messenger molecules (Nicholson and Calvert, 2010). H_2S is produced from cysteine by two enzymes, cystathionine gamma lyase (CSE) and cystathionine beta synthase (CBS) (Hosoki et al., 1997; Chen et al., 2004). CSE is found in many tissues, including the kidney (Hosoki et al., 1997). CSE activity is the predominant source of H_2S generation in the cardiovascular system (Wang, 2002). NO is produced by endothelial nitric oxide synthase (eNOS) (Brunner et al., 2001) and is known as a potent vasodilator (Ignarro et al., 1987). The CSE/H_2S (Zhao et al., 2001) and eNOS/NO (Moncada et al., 1991;

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Bredt and Snyder, 1992) pathways are both vasodilators and share the guanylyl cyclase/guanosine monophosphate pathway, which is operated by a G-protein coupled second messenger pathway system. The CSE/H_2S and eNOS/NO mediate the common guanylyl cyclase/guanosine monophosphate pathway for many of their actions and are supposed to stimulate each other's production. The interlinked production of H_2S and NO has been a topic of interest (Hosoki et al., 1997; Wang, 2002). The adrenergic receptors also operate via the G-protein coupled pathway (Guimarães and Moura, 2001). Hence, downregulation of the vasodilation CSE/H_2S and eNOS/NO pathways may also downregulate adrenergic receptors. The CSE/H_2S and eNOS/NO pathways, being vasodilators, would be expected to modulate the vasoconstriction mediated by noradrenaline released from sympathetic nerves or angiotensin II, as associated with the I/C model of LVH.

Pharmacological and molecular studies have documented three subtypes of α_1 adrenergic receptors: α_{1A}, α_{1B}, and α_{1D} (Hieble et al., 1995; Han et al., 2003), all from a G-protein coupled family (Guimarães and Moura, 2001). In the renal vasculature, α_{1A} adrenoceptors are reported to be the major subtype contributing to the constriction of the resistance vessels, intralobular arteries, and afferent and efferent arterioles (Sattar and Johns, 1994). The functional importance of this subtype has been evaluated in many pathophysiological states such as fructose-induced hypertension (Abdulla et al., 2011), stroke-prone hypertensive rats, the two-kidney one-clip model, and deoxycorticosterone acetate-salt hypertensive rats. In the aforementioned studies, renal vasoconstriction is mediated either by α_{1A} (Sattar and Johns, 1994a, 1994b; Villalobos-Molina and Ibarra, 1996), or by both α_{1A} and α_{1D} adrenoceptor subtypes (Villalobos-Molina et al., 1997; Salomonsson et al., 2000). Although reduced responsiveness of α_{1D} adrenoceptors in LVH was reported in our previous study (Ahmad et al., 2014), data are still lacking on the situation within the renal vasculature in LVH. However, there have been reports of α_{1A} adrenoceptors being involved in heart failure, an advanced stage of LVH, and diabetic Sprague–Dawley rats (Abbas et al., 2007).

We hypothesized that increased sympathetic activity due to induction of LVH in the I/C model and the consequent vasoconstriction due to raised plasma levels of noradrenaline and angiotensin may downregulate the vasodilatory CSE/H_2S and eNOS/NO pathways in the kidney and depress the α_{1A} adrenergic responsiveness to exogenously applied adrenergic agonists.

2. Materials and methods

Male Wistar Kyoto (WKY) rats (240 ± 10 g) were obtained from the animal house of Universiti Sains Malaysia, kept for 5 days of acclimatization in the animal transit room, and given free access to tap water and standard chow (Gold Coin Sdn. Bhd., Penang, Malaysia). These animals were randomly divided into four groups for 2 sets of experiments. One set consisted of control WKY and LVH WKY rats for vasoconstrictor study (n = 6), and the second set consisted of the same groups for molecular expression of CSE and eNOS mRNAs (n = 9) in the cortex part of the kidneys. LVH was induced by a modification of an earlier model using 5 injections of isoprenaline (5 mg/kg subcutaneously) on days 1, 4, 7, 10, and 13, and caffeine was given in the drinking water (62 mg/L), as reported by Ahmad et al. (2014). This approach is similar to a previously reported model (Flanagan et al., 2008), where only 4 injections of the same dose of isoprenaline were given. The control group was given 5 injections of saline within the same time intervals as the LVH group. This model and experimental procedure was approved by the Animal Research and Service Centre of Universiti Sains Malaysia with approval no./ 20 1 2 / (76) (364).

2.1. Chemicals

2.1.1. Agonists

Noradrenaline (Sanofi Winthrop, Surrey, UK) is a nonselective α adrenergic agonist that acts on α_1 and α_2 adrenergic receptors. Phenylephrine (Knoll, Nottingham, UK) has the ability to act nonselectively on α_{1A}, α_{1B}, and α_{1D} (Armenia et al., 2004). Methoxamine (Wellcome, London, UK) is relatively selective for α_{1A} adrenoceptors (Arévalo-León et al., 2003; Armenia et al., 2004) and can activate α_{1D}, but cannot differentiate between α_{1A} and α_{1D} (Arévalo-León et al., 2003).

2.1.2. Antagonists

The antagonist 5-methylurapidil (5-MeU; Research Biochemicals International, Natick, MA, USA) is a selective antagonist for the α_{1A} adrenoceptor subtype (Gross et al., 1988).

2.2. Quantification of CSE and eNOS mRNAs using the StepOnePlus RT-PCR system

After cervical dislocation, the kidney tissue was immediately preserved in RNAlater Solution (Ambion, Life Technologies, Pleasanton, CA, USA). Extraction was performed in a contamination-free area cleaned with RNaseZap (Ambion, Life Technologies). Total RNA was extracted from kidney tissue using TRIzol reagent (Ambion, Life Technologies) according to the manufacturer's guidelines. After the various sequential steps of homogenization, washing, and elution, total RNA was extracted, optimized, and quantified for purity and yield respectively using a microplate reader (Bio-Tek Instruments Inc., Winooski, VT, USA). Total RNA was converted to cDNA using a High Capacity RNA-to-cDNA kit (Applied Biosystems, Waltham, MA, USA) according

to the manufacturer's instructions. In this step, a 20-µL volume was used for the conversion of RNA to cDNA. Of the 20 µL, 11 µL comprised kit components (2X buffer, 10 µL; 20X enzymes, 1 µL), and the remaining 9 µL consisted of total RNA (depending upon the yield). RNAse-free water was used. The conversion of cDNA was performed using the default settings of the StepOnePlus RT-PCR system (Applied Biosystems) for this procedure.

TaqMan primers and probes for the CSE gene (GenBank Accession Nos. NM_017074.1 and Rn00567128_m1) were derived from TaqMan-Gene Expression assays (Applied Biosystems) (Hassan et al., 2012). TaqMan primers and probes for the eNOS gene (GenBank Accession Nos. NM_021838.2 and Rn02132634_s1) were derived from TaqMan-Gene Expression assays (Applied Biosystems) (Lee et al., 2013; Xu et al., 2013). Similarly, TaqMan primers and probes for the β-actin gene (GenBank Accession Nos. NM_031144.2 and Rn00667869_m1) were derived from TaqMan-Gene Expression assays (Applied Biosystems) (Cannino et al., 2009; Sántha et al., 2012).

The following primers for CSE, eNOS, and the internal control β-actin were used along with TaqMan chemistry (assay IDs: Rn00567128_ml, Rn002132634_s1, and Rn00667869_ml, respectively) for gene expression assays. TaqMan-Gene Expression assays were obtained and the procedure was followed according to the instructions of the manufacturer (Applied Biosystems). The amplification reaction consisted of a total of 20 µL of reaction mixture. One RT-PCR reaction consisted of 10 µL of TaqMan Fast Advanced Master Mix (2X) (Applied Biosystems); 1 µL of TaqMan Gene Expression assays (20X) of the respective genes CSE, eNOS, and beta-actin (Applied Biosystem); 8 µL of RNase-free water (Invitrogen, Carlsbad, CA, USA); and 1 µL of unknown sample cDNA. As a negative control of all the reactions, distilled water was added instead of cDNA. Temperature settings for RT-PCR were followed as default settings by the manufacturer (StepOnePlus RT-PCR, Applied Biosystems). Special MicroAmp Fast 96-well reaction plates (0.1 mL) (Applied Biosystems, Life Technologies) were used in the RT-PCR for amplification.

Quantitative RT-PCR reactions were carried out on 3 experimental animals and each rat was further analyzed in triplicate using the cortex of the kidney. Amplification of the housekeeping enzyme (internal control) β-actin allowed sample loading and normalization to be determined. For the relative quantification of target genes CSE, eNOS, and internal control β-actin, the comparative C_T (threshold cycle) method with the arithmetic formula ($2^{-\Delta\Delta CT}$) was used (Livak and Schmittgen, 2001).

2.2.1. Western blot analysis for CSE protein

CSE protein was determined with the method adopted previously (Zhu et al., 2010). Tissues were homogenized in the presence of lysis buffer consisting of 60 mmol/L Tris-HCl, 2% sodium dodecyl sulfate (SDS), 10% sucrose, 2 mmol/L phenylmethylsulfonyl fluoride (Merck, Darmstadt, Germany), 1 mmol/L sodium orthovanadate, and 10 µg/mL aprotinin (Bayer, Leverkusen, Germany). The cell lysates were quickly sonicated and centrifuged at 12,000 × *g* for 5 min at 4 °C. The supernatant was collected and the protein concentration was assayed using a modified Bradford assay. The samples were diluted in sample buffer (250 mmol/L Tris HCl (pH 6.8)) containing 4% SDS, 10% glycerol, 2% β-mercaptoethanol, and 0.002% bromophenol blue and were boiled for a further 5 min. Aliquots of proteins were separated by SDS-PAGE (10%) and subsequently transferred to nitrocellulose membranes by electroblotting. The membrane was blocked in 5% skimmed milk powder in 0.1% Tris-buffered saline/Tween 20 (TBST) at room temperature for 2 h and then incubated with antibody raised against CSE at a dilution of 1:2000 overnight at 4 °C. After three washes with TBST, the membrane was incubated with a secondary horseradish peroxidase-conjugated antibody for 1 h at room temperature. Immunoreactive proteins were visualized using the enhanced chemiluminescence western blotting detection system. The light-emitting bands were detected with X-ray film. The resulting band intensities were quantified using an image-scanning densitometer (Furi Technology, Shanghai, China).

2.2.2. Nitric oxide synthase (NOS) protein analysis

Frozen hearts were homogenized in a buffer of 50 mmol/L Tris-HCL (pH 7.4), 1 mmol/L EGTA, 1 mmol/L dithiothreitol, 1 mmol/L pepstatin A, 2 mmol/L leupeptin, and 1 mmol/L methanesulfonyl fluoride. All the homogenates were centrifuged at 10,000 × g at 4 °C for 60 min. Supernatant was taken as cytosolic fraction. All the pellets were solubilized by buffer containing 10% glycerol and 20 mmol/L 3-[(3-cholamidopropyl) dimethylammonio]-1-propanesulfonate and were ultracentrifuged to extract the particular fraction.

About 150 g of protein samples were separated from a particular fraction on 7.5% SDS-polyacrylamide gel, which was transferred to polyvinylidene difluoride membrane. About 5% nonfat dry milk was used to block the blots and they were incubated with rabbit polyclonal antibovine eNOS antibody (Ohashi et al., 1998; Ozaki et al., 2002). Immunoreactive bands were visualized with horseradish peroxidase-conjugated antirabbit IgG using an ECL detection kit.

NOS enzyme activity was determined by the conversion of [^{3}H]-L-arginine to [^{3}H]-L-citrulline with saturating concentrations of substrate and cofactors (Ozaki et al., 2002). Enzyme activity was expressed as citrulline production in femtomoles per milligram of protein per minute.

2.3. In vivo acute experiments for renal vasoconstrictor responses

An in vivo acute renal vasoconstrictor response experiment was conducted using previously published protocols (Abdulla et al., 2011). After overnight fasting, animals were anesthetized with 60 mg/kg i.p pentobarbitone (Nembutal, CEVA, Libourne, France). The electrocardiographic electrodes were attached and a tracheotomy was performed (PE240, Portex) using a midline incision. The right carotid artery was cannulated (PE50, Portex) and attached to a fluid-filled pressure transducer (P23 ID Gould; Statham Instruments, Nottingham, UK), whose output was connected to a data acquisition PowerLab (ADInstruments, Sydney, Australia) for continuous monitoring of blood pressure and heart rate. The left jugular vein was cannulated (PE50, Portex) for infusion of saline and maintenance doses of anesthetic. The aorta and left kidney were exposed using a midline abdominal incision. A laser Doppler flow probe (OxyFlow Probe; Oxford Optronix Ltd., Oxford, UK) was placed on the surface of the kidney to measure renal cortical blood perfusion (RCBP) throughout the experiment (Roman et al., 2001) and was connected to a laser Doppler flow meter (ADInstruments). The left iliac artery was cannulated (PE50, Portex) and the cannula was inserted into the aorta until it reached a point adjacent to the entrance of the renal artery. This cannula allowed the infusion of saline noradrenaline (NA), phenylephrine (PE), and methoxamine (ME) locally into the kidney. The saline was infused at 3 mL/h and the cannula was attached to a fluid-filled pressure transducer (P23 ID Gould; Statham Instruments). The output of the pressure transducer was attached to the PowerLab data acquisition system (PowerLab, ADInstruments). The bladder was cannulated for free urination. At the end of the surgery, the animals were allowed to stabilize for 1 h to ensure stable readings of systolic blood pressure (SBP), mean arterial pressure (MAP), heart rate (HR), and RCBP.

2.3.1. Renal vasoconstrictor responses

The three adrenergic agonists NA, PE, and ME were administered close renal arterially in ascending and descending dose order. The responses of these agonists in ascending and descending dose order were averaged to get a mean % drop in RCBP. NA was used at 25, 50, 100, and 200 ng; PE was used at 0.25, 0.5, 1, and 2 μg; and ME was administered at 1, 2, 3, and 4 μg. These doses were freshly prepared in saline solution every day and stored at 4 °C (Hieble et al., 1995). Each experiment consisted of three phases: a saline phase, and the low- and high-dose antagonist phases (5-MeU). In the saline phase, saline was administered intrarenally at a dose of 6 mL kg^{-1} h^{-1}. During this phase, all three agonists were administered in their ascending order and then descending order. Between each dose, agonists were washed out for 12 min for complete removal of the previous dose of agonist (Abdulla et al., 2011). In the low-dose phase of antagonist, 5-MeU was administered intrarenally as a bolus dose of 5 μg/kg and then as a maintenance infusion of 1/4 of the bolus dose (1.25 μg kg^{-1} h^{-1}). In the low-dose phase, the doses of each agonist were administered in the same pattern as in the saline phase. In the high-dose phase, 5-MeU was administered intrarenally as a bolus dose of 10 μg/kg intrarenally, followed by a maintenance dose of 1/4 of the bolus dose (2.5 μg kg^{-1} h^{-1}). The doses of the agonists followed the same pattern as in the saline and low-dose phases. At the end of the acute vasoconstrictor study, the animals were euthanized with pentobarbital.

2.3.2. Measurement of heart, LV, and kidney indices

After the completion of the acute experiment, the heart and contralateral kidney were carefully extracted and dried on blotting paper to prevent the interference of blood in the weight measurement. The heart and kidneys were weighed carefully, and then the LV was isolated from the whole heart and carefully weighed. These values were used to generate the heart, kidney, and LV indexes as follows:

Heart index = heart weight / body weight × 100

Left ventricle index = left ventricle weight / body weight × 100

Kidney index = kidney weight / body weight × 100

2.4. Measurement of H_2S and NO concentrations in plasma

A blood sample was taken from the tail artery on day 14 and was centrifuged at 5000 × *g* for 10 min (Ahmad et al., 2014). The plasma was separated and used for the estimation of H_2S and NO. The plasma concentration of nitric oxide was measured using kits, according to the manufacturer's instructions (NJJC Bio Inc., Nanjing, China). The plasma concentration of H_2S was measured as reported previously (Yan et al., 2004; Ahmad et al., 2012).

2.5. Statistical analysis

In this study, the statistical analysis for vasoconstriction was undertaken using two-way analysis of variance (ANOVA), followed by a Bonferroni post hoc test and an independent Student t-test for physical parameters of the heart, systemic hemodynamic parameters, concentration of H_2S and NO, and expression of CSE and eNOS mRNAs. Overall mean % drop in RCBP bar graphs was analyzed by using one-way ANOVA followed by the Bonferroni post hoc test using GraphPad Prism (GraphPad Software, San Diego, CA, USA). All data were expressed as mean ± SEM with significance at $P < 0.05$.

3. Results

3.1. Physical indices of heart and kidney

The kidney, heart, and LV indices were significantly elevated ($P < 0.05$) in the LVH WKY group compared to the control WKY rats (Table 1).

Table 1. Physical parameters of kidney, heart, and LV in control WKY and LVH WKY groups (n = 6).

	Control WKY	LVH WKY
Heart index (%)	0.27 ± 0.01	0.39 ± 0.01*
Kidney index (%)	0.29 ± 0.00	0.34 ± 0.017*
LV index (%)	0.15 ± 0.01	0.24 ± 0.001*

WKY: Wistar Kyoto rats; LVH: left ventricular hypertrophy.
*P < 0.05 compared to the control.

3.2. Systemic hemodynamic data in control WKY and LVH WKY groups

SBP and MAP were significantly elevated and HR was reduced in LVH WKY compared to the control WKY (P < 0.05). RCBP was lower in LVH WKY compared to the control WKY group (P < 0.05), as shown in Table 2.

3.3. Measurement of H_2S and NO concentrations in plasma

The concentration of H_2S in the plasma of LVH WKY was significantly (P < 0.05) lower compared to the control WKY (LVH WKY 16 ± 2 vs. control WKY 37 ± 3 μM), as shown in Table 2. Similarly, the plasma concentration of NO was reduced significantly (P < 0.05) in LVH WKY compared to the control WKY (LVH WKY 21 ± 1 vs. control WKY 25 ± 2 μmol/mL).

3.4. Expression of renal CSE mRNA, eNOS mRNA, and respective proteins

Induction of LVH in WKY rats reduced the expression of renal CSE mRNA to 0.32-fold relative to that of the control WKY rats (P < 0.05). Similarly, the expression of renal eNOS mRNA was also reduced in LVH WKY rats by 0.21-fold compared to the control WKY rats (P < 0.05), as shown in Figures 1A and 1B. It can be seen by the visualized band that both CSE and eNOS proteins are reduced in the LVH group when compared to the control group, as shown in Figures 1C and 1D.

3.5. Renal vasoconstrictor responses

The adrenergic agonists NA, PE, and ME produced dose-dependent renal vasoconstrictions in both the control WKY and LVH WKY groups, as shown in Figures 2A–2F. The magnitude of the renal vasoconstrictor responses to NA, PE, and ME in the saline phase in the LVH WKY rats were significantly (P < 0.05) attenuated compared to the control WKY group (NA, 27 ± 2% vs. 43 ± 6%; PE, 32 ± 7% vs. 44 ± 3%; ME, 29 ± 1% vs. 40 ± 6%) when data were expressed as overall mean % drop in RCBP, as shown in Figures 3A–3C.

With the low dose of the antagonist phase, the renal vasoconstriction responses to NA and PE were not attenuated in the LVH group when a comparison was performed against the low-dose phase of the control WKY group. However, there was a significant attenuation of the responses of ME in the LVH WKY group as compared to the low-dose phase of the control WKY (NA, 25 ± 2% vs. 29 ± 6%; PE, 28 ± 4% vs. 34 ± 3%; ME, 22 ± 2% vs. 32 ± 8%; Figures 3A–3C).

Table 2. Baseline values of SBP, MAP, HR, and RCBP were measured during the acute experiment on day 14 for control WKY and LVH WKY groups (n = 6). *: Significance compared to LVH WKY (P < 0.05).

Parameters	Control WKY	LVH WKY
SBP (mmHg)	130 ± 7	160 ± 9*
MAP (mmHg)	116 ± 4	144 ± 9*
Heart rate (bpm)	314 ± 9	264 ± 18
RCBP (bpu)	157 ± 19	102 ± 5*
H_2S (μM)	37 ± 3	16 ± 2*
NO (μmol/mL)	25 ± 1.30	21 ± 1*

SBP: Systolic blood pressure, MAP: mean arterial pressure, HR: heart rate, bpm: beats per min, RCBP: renal cortical blood perfusion, bpu: blood perfusion unit, WKY: Wistar Kyoto rats, LVH: left ventricular hypertrophy, H_2S: hydrogen sulfide, NO: nitric oxide.

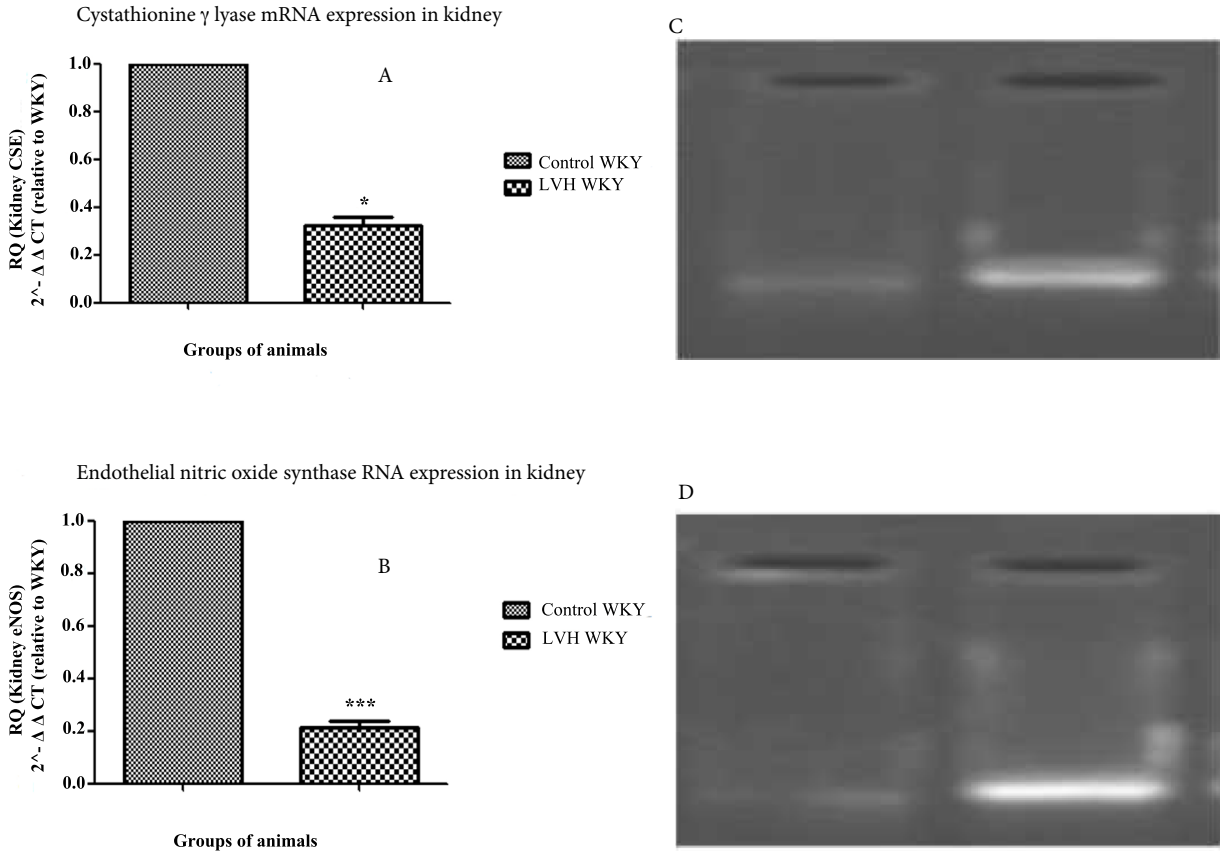


Figure 1. Relative quantification of (A) CSE mRNA and (B) eNOS mRNA expression in the kidney of the control WKY and LVH WKY. *: $P < 0.05$ compared to the control WKY group. The data are relative to the control WKY and normalized to internal control β-actin. (C, D) Both CSE and eNOS proteins are reduced in the LVH group when compared to the control group.

In the high-dose phase of antagonist, there was no attenuation of the renal vasoconstrictor response to NA in the LVH WKY group when compared to the same dose of the control WKY group (NA, 12 ± 2% vs. 18 ± 3%). However, at the same time, statistically significant attenuation ($P < 0.05$) was observed in the LVH WKY group when PE and ME were used in the high-dose phase of 5-MeU (PE, 13 ± 1% vs. 23 ± 5%; ME, 12 ± 3% vs. 21 ± 1%; Figures 3A–3C).

4. Discussion

This investigation aimed to determine the mechanisms contributing to renal vasoconstrictor responsiveness to adrenergic agonists in a pathophysiological model of LVH. The I/C treatment resulted in a marked increase in left ventricular weight, demonstrating that LVH had been induced; an increase in blood pressure, reflecting an increase in total peripheral resistance; and a decrease in RCBP, indicating elevated vascular resistance in the kidney. There were two major novel observations. First, there was a blunting of the renal vascular responsiveness to the adrenergic agonists, which appeared to be primarily mediated via the α_1 adrenoceptor subtype. Second, there was a marked depression in the expression of the CSE and eNOS genes in the kidney, suggesting that the generation of vasodilators H_2S and NO was reduced in this model of LVH. The question that arises is whether the reduction in H_2S and NO and its association with reduced vascular responsiveness is a direct one at the level of the signaling cascades, or whether it is indirect as a consequence of the increase in vascular tone.

After 2 weeks of treatment with caffeine and isoprenaline, there was a significant increase in cardiac and LV indices in the LVH WKY rats compared to the control WKY rats, which is in agreement with previous reports using this model (Flanagan et al., 2008). This increased heart weight is probably due to an increase in workload induced by isoprenaline acting on β_1 adrenoceptors at the sinoatrial node, which increases the heart rate and cardiac output, thereby resulting in hypertrophy of the left ventricle (Leenen et al., 2001). Nevertheless, the present study and several previous studies using this LVH model have shown a decrease in heart rate. The exact mechanism is still unknown, but it may be due to the anesthetic used

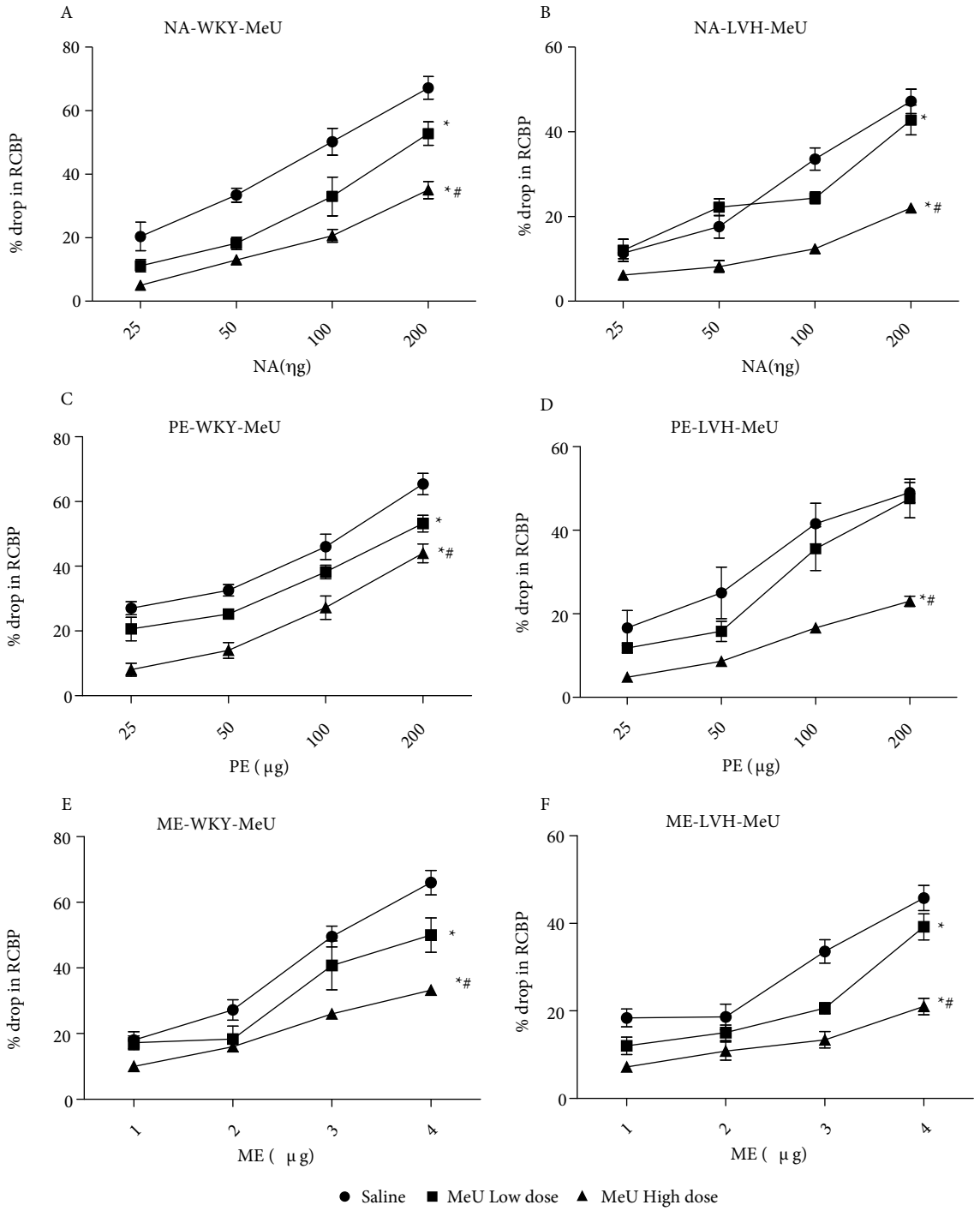


Figure 2. Line graph shows dose-dependent curves of the renal vasoconstrictor responses to the graded doses of adrenergic agonists. (A, B) NA, (C, D) PE, (E, F) ME in the control WKY and LVH WKY groups in saline and low- and high-dose phase 5-MeU. Values are mean ± SEM, n = 6 rats in each group. The significance is calculated as overall mean responses of four doses of each agonist during each phase and compared with the saline phase.*: $P < 0.05$ vs. saline phase.

in these studies. SBP and MAP were significantly elevated in the LVH group, which may be partly due to elevated renin and angiotensin II levels, which have been reported in this I/C model. Moreover, angiotensin II, acting either systemically (Leenen et al., 2001) or locally in the kidney (Crowley et al., 2006), will also contribute to blood pressure elevation and cardiac hypertrophy. Interestingly, our data are in contrast to previous studies using a similar model,

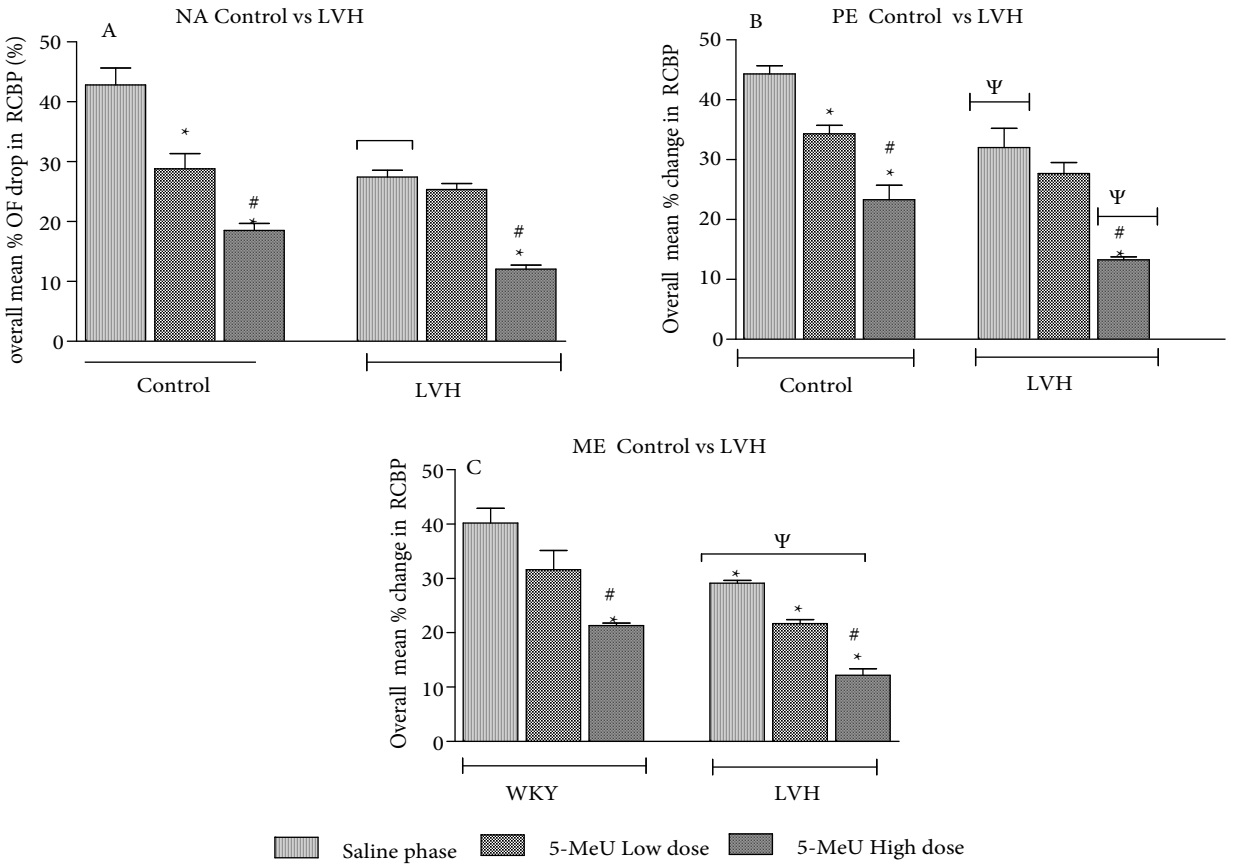


Figure 3. Bar graph shows % drop in overall mean of change in renal cortical blood perfusion in response to NA, PE, and ME in the presence and absence of 5-MeU antagonist in the control WKY and LVH WKY groups. Values are mean ± SEM, n = 6 rats in each group. The significance is calculated as overall mean responses of four doses of each agonist during each phase and compared with the saline phase. *: $P < 0.05$ vs. saline phase. #: $P < 0.05$ vs. low-dose phase of respective group. ψ: $P < 0.05$ vs. same phase of the control WKY group.

which found that MAP did not change after 2 weeks of treatment with isoprenaline and caffeine (Flanagan et al., 2008). This may be due to the modification undertaken in the model of the present study by administering 5 isoprenaline injections.

An important novel observation was that the induction of LVH resulted in a decrease in the expression of the CSE gene in the renal cortex of the kidney, which was probably responsible for the decreased plasma levels of H_2S. Although only the kidney was examined in this study, it is likely that a similar situation pertained to all other organs of the body. It is recognized that H_2S is a vasodilating factor, and its decreased intrarenal production would lead to an increased vascular tone and hence resistance within the kidney. The way in which H_2S exerts its action is unclear, but there is evidence that it induces relaxation of resistance vessels via activation of ATP-sensitive potassium channels (Hosoki et al., 1997). Thus, the reduction in the CSE/H_2S axis in LVH would contribute to elevated blood pressure and vascular resistance. A further novel finding was that, concomitantly, LVH caused a decrease in the expression of eNOS in the renal cortex kidney, which was reflected in the reduction in plasma NO concentrations. NO induces vasodilation via activation of guanylyl cyclase and therefore reduction in the eNOS/NO pathway would also lead to an increase in tone in all resistance vessels, including the kidney, as observed in the present study. There is evidence that NO acts primarily on the larger resistance vessels (Wilkinson et al., 2002). Together, the reductions in the CSE/H_2S and eNOS/NO pathways may be the primary factors responsible for the increased blood pressure and decreased renal cortical perfusion observed in this LVH model. Moreover, the downregulation of the CSE/H_2S and eNOS/NO pathways indicates some common pathway for the production of both gaseous transmitters, which needs to be elucidated by upregulating the one pathway and observing the production of H_2S and NO in future studies.

It was important to examine how the withdrawal of the vasodilator factors, H_2S and NO, would influence the ability

of the adrenergic agonists to cause vasoconstriction in the kidney. It was evident that there was a marked blunting of the renal vasoconstrictor actions of all the adrenergic agonists tested. The underlying reasons are unclear, but there are two possibilities: direct and indirect. The first possibility is that while the basal vascular tone within the kidney is substantially elevated in the LVH model, the ability of the agonists to increase the tone further would be limited, reflected as reduced responsiveness. A second possible mechanism is interference along the signaling pathways. Binding of the α_1 adrenoceptors to their G-coupled proteins activates a cascade, whose end point is an increase in intracellular calcium and opening of calcium channels. However, if the smooth muscle cells are already hyperpolarized as a consequence of the decrease in H_2S, then the ability of the alpha adrenergic compounds to induce depolarization and contraction will be decreased.

In the present study, LVH was associated with a lower RCBP compared to the control WKY, as shown in Table 2. The downregulation of the vasodilator CSE/H_2S pathway may be one of the reasons for RCBP, as exogenous H_2S supply improves the RCBP in spontaneously hypertensive rats (Ahmad et al., 2014). Another possibility is the downregulation of the eNOS/NO pathway associated with LVH induction. Decreased plasma NO concentration would lead to an increased vascular tone, which would be expressed in the kidney as decreased RCBP. A further factor contributing to reduced RCBP would be the increased plasma levels of NA and angiotensin II caused by the activation of the sympathetic nervous system (Collomp et al., 1991; Bell et al., 2001).

The attenuation of the vascular responses to the adrenergic agonists NA, PE, and ME were significantly blunted in the saline phase as well as with the low and high doses of 5-MeU in the LVH WKY group compared to control WKY. These attenuated responses to adrenergic agonists may be due to elevated plasma levels of NA and angiotensin II, as reported previously with this model (Bell et al., 2001; Crowley et al., 2006). Our data are in agreement with other pathophysiological studies, in which elevated levels of circulating NA and angiotensin II reduced the responsiveness of adrenergically mediated vasoconstriction (Tran et al., 2009; Abdulla et al., 2011).

The reduced renal vasoconstrictor responses to NA, PE, and ME during the saline phase of LVH WKY indicate that α_1 adrenergic receptor signaling cascades and/or vascular responsiveness are attenuated, which may be due to the downregulation of the CSE/H_2S and eNOS/NO pathways. The blockade of α_{1A} adrenergic receptors by the low-dose 5-MeU in the control WKY group blunted the responses to NA and PE but did not affect the responses of NA and PE in LVH WKY, indicating that adrenergic receptors' responsiveness to these nonselective α_{1A} adrenoceptor agonists was altered by the induction of LVH (Khan et al., 2008). By contrast, the responses to ME, a relatively selective agonist (Arévalo-León et al., 2003), were unchanged in the WKY and LVH groups. However, the partial or complete blockade of α_{1A} adrenergic receptors with 5-MeU attenuated the responses of the selective α_{1A} adrenergic receptor agonist. These findings suggest that it is the α_{1A} adrenoceptor subtype that is responsible for the modulation of vasoconstrictor responses in LVH, and the magnitude of these responses is attenuated with the induction of LVH.

In conclusion, these findings suggest that the downregulation of the CSE/H_2S and eNOS/NO pathways in the kidney and the blunted responses to the α_{1A} adrenergic agonists were consistent with an alteration in the G-protein second messenger coupled pathway due to the induction of LVH. Therefore, the upregulation of both vasodilatory pathways is expected to ameliorate the blunted responses of the α_{1A} adrenergic receptor.

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