Elevated sympathetic activities, hyperactivity of the heart are usually associated with the establishment of left ventricular hypertrophy (LVH). The increase in elevated sympathetic activity is proportional to the increase in mass of LV (1). This increase in sympathetic activity is blamed to have impact on vascular functions and impairs the renal vasoconstrictions mediated by \( \alpha_1 \) adrenoreceptor (2). The functional contribution of \( \alpha_1 \) adrenergic receptors in different disease states with sympathetic hyperactivity has been studied (3-6). The isoprenaline and caffeine model (I/C model) of LVH is reported for elevated plasma levels of noradrenaline (NA) and angiotensin II (6-8). LVH has been found to have significant impact on renal vascular responses to adrenergic stimuli and \( \alpha_1 \) have been reported as contributing factor in modulating the vascular responses (6).

There are three \( \alpha_1 \)-adrenergic receptors \( \alpha_{1A}, \alpha_{1B} \) and \( \alpha_{1D} \) (9). Among these three receptors, \( \alpha_{1B} \) have not been given high priority as compared to \( \alpha_{1A} \) and \( \alpha_{1D} \). It has been observed that \( \alpha_{1B} \) have been modulating the renal vasoconstriction in normal Sprague-Dawley rats and fructose feeding did not influence the functionality of \( \alpha_{1B} \) adrenergic receptors in mediating the renal vasoconstriction (10) and \( \alpha_{1B} \) mediates the renal vasoconstriction in rats with renal impairment but not in the rats with normal renal function (11). It has been observed that high sodium diet in SHR has no major influence on the functionality of \( \alpha_{1B} \) adrenergic receptors (12). Among the known pool of \( \alpha_1 \)-adrenergic receptors, expression of mRNA of \( \alpha_{1B} \) have been much lower than the \( \alpha_{1A} \) but much greater than \( \alpha_{1D} \) (13). The role of \( \alpha_{1B} \) mediating the constriction of smooth blood vessels is less certain and only few studies reported that \( \alpha_{1B} \) is

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**Keywords:** left ventricular hypertrophy, endothelial nitric oxide synthase, \( \alpha_{1B} \) adrenergic receptor
involved in the contraction of blood vessels (14). The \( \alpha_{1B} \) was earlier supposed to cause contractions of aorta (15). However, recent studies have used BMY7378 and reported that \( \alpha_{1D} \) is involved in regulating the contractions (13, 16). Both \( \alpha_{1B} \) and \( \alpha_{1D} \) exhibit similar kind of affinity for few ligands used for receptors characterization (17). In our previous study (6), we observed that responsiveness of \( \alpha_{1D} \) adrenergic receptor subtypes is blunted to adrenergic agonist. So far, no data is available to claim that this uncertain role of vasoconstriction is mediated by \( \alpha_{1B} \) in kidney in LVH. We proposed renal vasoconstriction responses to adrenergic stimuli, blunted in LVH and \( \alpha_{1B} \), being more expressed than \( \alpha_{1D} \) and similar in responses to various agonist as \( \alpha_{1D} \). are modulating the renal vasoconstriction in this model of LVH.

The decrease in vasoconstriction responses are believed to be either down regulation of mRNA expression or desensitization of the receptors (2, 18) due to hyperactivity of sympathetic nervous system. This hyperactive sympathetic system elicits physiological responses by G-protein coupled adrenergic receptors (19) which use guanylyl cyclase pathway. Increased vasoconstriction either due to raised noradrenaline and angiotensin levels in plasma in I/C model of LVH (6) is expected to down regulate the counter regulatory vasodilation phenomenon in either systemic circulation or in vascular tissue. This LVH pathophysiology is blamed to be nitric oxide deficient model and may be one of the reasons for dominant vasoconstriction pathways in LVH. Nitric oxide is produced by endothelial nitric oxide synthase (eNOS) (20, 21) and is known as a potent vasodilator (22). Thus, eNOS/NO pathways generate vasodilators and share guanylyl cyclase/guanosine monophosphate pathway which is operated by a G-protein coupled second messenger pathway system. So, we are of the view that the kidney of LVH rat is imbalanced by down regulation of endothelial nitric oxide synthase (eNOS), which may lead to decreased production of NO locally and consequently local elevation of NA and angiotensin. We also proposed that angiotensin may lead to elevated oxidative stress in systemic circulation and in the kidney tissue, which may be another contributing factor in attenuation of renal vascular responses by \( \alpha_{1B} \) adrenergic receptor.

Although the impact of \( \alpha_{1B} \) in mediating the renal vasoconstriction in LVH is uncertain but we are of the view that renal vasoconstriction responses are blunted in LVH and \( \alpha_{1B} \) is contributing factor in the regulation of renal vasoconstriction in LVH. We also propose that this attenuation in the responses of \( \alpha_{1B} \) may be due to the increase in oxidative stress and down regulation of eNOS/NO pathway in the kidney.

**MATERIALS AND METHODS**

**Animals**

Male Wistar Kyoto (WKY) rats aged between 10-12 weeks were obtained from the animal house of Universiti Sains Malaysia. Animals were acclimatized for 5 days and given continuous supply of tap water and standard chow (Gold Coin Sdn. Bhd., Penang, Malaysia). The rats were divided into two sets of groups. One set consisted of Control WKY and LVH-WKY groups each having \( n = 6 \) for acute renal study and other set consisted of the same groups but for molecular expression of endothelial nitric oxide synthase (eNOS) having 3 biological samples having \( n = 9 \). LVH was induced by administration of isoprenaline (5 mg/kg s.c., 24 h apart) and caffeine 62 mg/L in drinking water for 14 days as reported (6). The corresponding Control WKY were given saline s.c. and tap water instead of isoprenaline and caffeine and kept for same period of time. Experimental procedure was approved from Animal Research and Service Center (ARASC) in Universiti Sains Malaysia.

**Chemicals**

**Agonists**

Noradrenaline (Sanofi Winthrop, Surrey, UK), methoxamine (Wellcome, London, UK), are non selective \( \alpha \) adrenergic agonists which acts on \( \alpha_1 \) and \( \alpha_2 \) adrenergic receptor; phenylephrine (Knoll, Nottingham, UK) has the ability to act nonselectively on \( \alpha_{1A}, \alpha_{1B} \) and \( \alpha_{1D} \) (3); methoxamine is relatively selective for \( \alpha_{1A} \) adrenoceptors (3) and can activate \( \alpha_{1D} \) but can not differentiate between \( \alpha_{1A} \) and \( \alpha_{1D} \).

**Antagonists**

The antagonist - chloroethylclonidine (Research Biochemicals International, Natick, MA, USA) is a selective antagonist for the \( \alpha_{1B} \) adrenoceptor subtype (23).

**Measurements**

**Measurement of angiotensin converting enzyme activity in plasma**

The hippuryl-L-histidyl-L-leucine (HHL) substrate was prepared freshly at 10 mM in phosphate buffer pH 8.3 (0.2 M potassium phosphate anhydrous and 0.3 M sodium chloride, and the pH was adjusted to 8.3 by adding 3.0 M potassium hydrox-
Increased oxidative stress and down regulation of endothelial nitric...

The substrate solution was sonicated for 30 min until HHL is totally dissolved. ACE stock solution from rabbit lung was prepared at 200 mU/mL in the phosphate buffer pH 8.3 that contains 0.1% bovine serum albumin (BSA), and further diluted in the same buffer. The blood samples were collected in heparinized tubes and plasma was obtained by centrifugation at 3000 rpm for 5 min and saved at -20°C until used. The reaction was carried out by adding 25 µL of ACE standard or plasma samples to 175 µL HHL, and incubating at 37°C for 2 h. Subsequently, the reaction was stopped by heating at 80°C for 15 min, and the samples were diluted 1:5, v/v with distilled water. The concentration of hippuric acid, the end product of CAE degradation of HHL, was measured by reverse HPLC as described (24). A calibration curve of ACE activity (0.0016 ñ 0.05 U/mL) was prepared at the same time, and the regression equation (y = 5949.4x + 79.9, \( R^2 = 0.9721 \)) was applied to calculate the ACE activity in the plasma samples.

**Measurement of oxidative stress in plasma and kidney tissue**

Oxidative stress markers: malondialdehyde (MDA), superoxide dismutase (SOD) and glutathione levels in the plasma and kidney were quantified by using the information provided by instruction (NJJC Bio Inc., Nanjing, China). In the kidney tissue homogenate the concentration of protein was estimated by using the reported method (25).

**Measurement of plasma and kidney tissue nitric oxide concentration**

The concentration of nitric oxide was determined by using kits (NJJC Bio Inc., Nanjing, China) on day 14th and following the instruction provided by manufacturer. Procedure involves preparation of blank, standard solution and assay solution. For assay solution, 100 µL of plasma sample was mixed with 400 µL of working solution by using vortex. The resultant solution was kept at 37°C in thermostat water bath for 60 min. The reagent 3 was added (200 µL) followed by100 µL of reagent 4. The whole solution was vortexed for 30 s and was allowed to stand at room temperature for 40 min, then was centrifuged at 4000 rpm for 10 min. About 500 µL of supernatant was removed carefully without disturbing the precipitate. The developer (600 µL) was added, the mixture was vortexed and allowed to stand at room temperature for 10 min. Absorbance was measured at 550 nm. The NO amount was calculated by using equation:

\[
\text{NO (µmol/L) = } \frac{\text{OD of assay} - \text{OD of blank} \times \text{Standard conc. (100 mmol/L)} \times \text{D.F.}}{\text{Standard conc./protein conc. (g/L)}}
\]

where OD: optical density, D.F.: dilution factor.

**Acute renal vasoconstrictor study**

*In vivo* acute renal vasoconstrictor study was performed by following the procedure already reported (4, 6). Anesthesia was induced by administration of an intraperitoneal injection of sodium pentobarbitone (Nembutal, CEVA) 60 mg/kg. After complete onset of anesthesia, a blunted incision was made at the neck region to facilitate the breathing throughout the experiment. After tracheotomy, carotid artery was cannulated (Portex, Kent, UK) and was attached to pressure transducer (Gould P23 ID; Statham Instruments) further attached to PowerLab data acquisition system or continuous monitoring of systemic hemodynamics. The left jugular vein was also cannulated (Portex, Kent, UK) for infusion of saline and maintenance doses of anesthesia on need basis. The left kidney was exposed by a midline incision with cautery and a laser Doppler flow probe (ADInstruments) was placed on the surface top of cortex region of the kidney to measure renal cortical blood perfusion. The iliac artery was cannulated (Portex, Kent, UK) and PWV was measured by measuring the propagation time (t) from carotid artery to iliac artery from PowerLab data and propagation distance was measured manually by putting a thread from insertion point of carotid artery to insertion point of iliac artery (26, 27). Cannula was pushed at the position of renal artery in such a way that administration of adrenergic agonist like noradrenaline (NA), phenylephrine (PE) and methoxamine (ME) directly reach to the kidney (6, 28, 29). Animal was allowed to stable for 1 h before the onset of vasoconstrictor part of the study.

**Acute vasoconstrictor study**

NA, PE and ME were administered intra-renal-ly in increasing and decreasing sets of doses in such a way that net response was calculated as average of the increasing and decreasing doses. NA was given in 25.0, 50.0, 100 and 200.0 ng; PE was adminis-
tered as 0.25, 0.50, 1.0 and 2.0 µg and ME was administered as 0.25, 0.50, 1.0 and 2.0 µg as reported (6). These solutions were prepared on daily basis. Experiment was divided into 3 phases consisting of saline phase, CEC low dose phase and CEC high dose phase. In saline phase, saline was infused in the kidney at the rate of 6 mL/kg/h and in low dose phase CEC was induced at the dose of 5 mg/kg as bolus dose followed by 1/4th of maintenance dose along with ascending and descending doses of adrenergic agonists. In high dose phase, CEC was administered as 10 mg/kg as bolus dose and followed by maintenance dose along with ascending and descending doses of adrenergic agonists.

Quantification of endothelial nitric oxide synthase (eNOS) expression in kidney by using StepOnePlus RT-PCR system.

Protocol of study was adopted as reported (30). However, the description is explained. After the cervical dislocation of the rats, cortex part of the kidney tissue was immediately preserved in RNAlater® Solution (Ambion, Life Technologies, USA). Extraction was carried out in an area cleaned by RNaseZap® (Ambion, Life Technologies, USA). Animal total RNA was extracted from kidney tissue by using TRIzole reagent (Ambion, Life Technologies, USA) according to manufacturer guidelines. After the various sequential steps of homogenization, washing and elution, total RNA was extracted from kidney tissue by using TRIzole reagent (Ambion, Life Technologies, USA) according to manufacturer’s instruction. In this step total 20 µL volume was used for the conversion of RNA to cDNA. Out of 20 µL, 11 µL kit components (2 × buffer 10 µL, 20× enzyme 1 µL) and remaining 9 µL consisted of total RNA (depending upon the yield) and RNase free water were used. Conversion of cDNA was done by using default settings for this procedure of StepOnePlus RT-PCR (Applied Biosystems, Singapore).

TaqMan primers and probes for eNOS (Gen Bank accession No. NM_021838.2 and Rn02132634_s1) gene was derived from TaqMan® Gene Expression assays (Applied Biosystems, USA) (31, 32). Similarly, TaqMan primers and probes for β-actin (Gen Bank accession No. NM_031144.2 and Rn00667869_m1) gene was derived from TaqMan® Gene Expression assays (Applied Biosystems, USA) (33, 34).

Following primers for eNOS and internal control β-actin along with Taqman chemistry were used (assay ID: Rn002132634_s1 and Rn00667869_mL, respectively) for gene expression assay. TaqMan® Gene Expression Assays were obtained and the procedure was followed according to the instructions of manufacturer. Amplification reaction consists of total 20 µL volume of reaction mixture. One RT-PCR reaction consists of 10 µL of TaqMan® Fast Advanced Master Mix (2×) (Applied Biosystem™, USA), 1 µL of TaqMan® gene Expression Assays (20×) of respective gene CSE, eNOS and β-actin (Applied Biosystem™, USA), 8 µL of RNase Free Water (RFW) (Invitrogen™, 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. Special MicroAmp® Fast 96-well Reaction Plate (0.1 Ml) (Applied Biosystems, Life Technologies, USA) for amplification were used in RT-PCR.

Quantitative RT-PCR reactions were carried out in triplicate for each rat. Amplification of house-keeping enzyme (internal control) β-actin was used for each sample loading and normalization. The relative quantification of target gene eNOS and internal control β-actin, the comparative C<sub>T</sub> (threshold cycle) method with arithmetic formula (2<sup>-∆∆C\text{T}</sup>) was used (35).

Table 1. Oxidative stress parameters in the plasma of Control WKY and LVH-WKY on final day of experiment. Data presented as the mean ± SEM. p < 0.05. * p < 0.05 vs. Control WKY.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control WKY</th>
<th>LVH-WKY</th>
</tr>
</thead>
<tbody>
<tr>
<td>MDA (nmol/mL)</td>
<td>20.67 ± 2.35</td>
<td>36 ± 0.4*</td>
</tr>
<tr>
<td>GSH (µmol/mL)</td>
<td>542 ± 17</td>
<td>134 ± 19*</td>
</tr>
<tr>
<td>SOD (U/mL)</td>
<td>5.5 ± 0.4</td>
<td>3 ± 0.6*</td>
</tr>
<tr>
<td>NO (µmol/mL)</td>
<td>28 ± 1</td>
<td>20 ± 1*</td>
</tr>
<tr>
<td>ACE activity (U/mL)</td>
<td>0.10 ± 0.01</td>
<td>0.35 ± 0.02</td>
</tr>
</tbody>
</table>
Increased oxidative stress and down regulation of endothelial nitric...

Statistical analysis

Acute renal vasoconstrictor study was analyzed by using 2 way ANOVA followed by Bonferroni post hoc test while Student t test was also applied to parameters like oxidative stress measurement, concentration of nitric oxide and ACE assay by using GraphPad Prism (GraphPad Software, California, USA) with values for p < 0.05 statistically considered significant. Molecular expression for eNOS was analyzed by using arithmetic formula (2^−ΔΔCT).

RESULTS

Measurement of angiotensin converting enzyme activity in plasma

The ACE activity in LVH-WKY was increased as compared to Control WKY (Control WKY vs. LVH-WKY: 0.10 ± 0.01 vs. 0.35 ± 0.02 U/mL) as shown in Table 1.

Measurement of oxidative stress in plasma and kidney tissue

Oxidative stress was assessed by using oxidative stress markers like MDA, SOD, and GSH in the plasma and kidney tissue. MDA levels were increased significantly (p < 0.05) in plasma of LVH-WKY group when compared to Control WKY (LVH-WKY vs. Control WKY (nmol/mL): 36 ± 0.4 vs. 20.67 ± 2.35) while SOD levels in the plasma were reduced significantly (p < 0.05) in LVH-WKY when compared to Control WKY (LVH-WKY vs. Control WKY (U/mL): 3 ± 0.6 vs. 5.5 ± 0.04 U/mL) as shown in Table 1. GSH levels in plasma of LVH-WKY were reduced significantly (p < 0.05) in LVH-WKY when compared to Control WKY (LVH-WKY vs. Control WKY (µmol/mL): 134 ± 19 vs. 542 ± 17 µmol/mL) as shown in Table 1.

Oxidative stress in the kidney tissue was also measured to correlate the oxidative stress in plasma. MDA levels were increased significantly (p < 0.05) in plasma of LVH-WKY group when compared to Control WKY (LVH-WKY vs. Control WKY (nmol/mL/mg): 95 ± 3 vs. 144 ± 4 while SOD levels in the plasma were reduced significantly (p < 0.05) in LVH-WKY when compared to Control WKY (LVH-WKY vs. Control WKY (U/mL/mg): 11 ± 1 vs. 5.0 ± 1 as shown in Table 2. GSH levels in plasma of LVH-WKY were reduced significantly (p < 0.05) in LVH-WKY when compared to Control WKY (LVH-WKY vs. Control WKY: 6 ± 1 vs. 3 ± 1 µmol/mL/mg) as shown in Table 2.

Measurement of plasma and kidney tissue nitric oxide concentration

Plasma level of NO was reduced significantly (p < 0.05) in LVH-WKY when compared to Control

Table 2. Oxidative stress parameters in the kidney of Control WKY and LVH-WKY on final day of experiment. Data presented as the mean ± SEM. p < 0.05. * p < 0.05 vs. Control WKY.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control WKY</th>
<th>LVH-WKY</th>
</tr>
</thead>
<tbody>
<tr>
<td>MDA (nmol/mL/mg)</td>
<td>95 ± 3</td>
<td>144 ± 4*</td>
</tr>
<tr>
<td>GSH (µmol/mL/mg)</td>
<td>6 ± 1</td>
<td>3 ± 1*</td>
</tr>
<tr>
<td>SOD (U/mL/mg)</td>
<td>11 ± 1</td>
<td>5 ± 1*</td>
</tr>
<tr>
<td>NO (µmol/g protein)</td>
<td>0.91 ± 0.03</td>
<td>0.27 ± 0.01*</td>
</tr>
</tbody>
</table>

Figure 1: Expression of endothelial nitric oxide synthase (eNOS) in kidney of Control WKY and LVH-WKY groups. The relative quantification was measured by using 2^−ΔΔCT method and data among two groups were analyzed by using Student t test using Graph Pad Prism. Values are presented as the mean ± SEM. p < 0.05. * p < 0.05 vs. Control WKY.
WKY (Control WKY vs. LVH-WKY (µM): 20 ± 1 vs. 28 ± 1, respectively) as shown in Table 1. Similarly, kidney tissue level of NO was reduced significantly (p < 0.05) in LVH-WKY when compared to Control WKY (Control WKY vs. LVH-WKY (µM/mg of protein): 0.91 ± 0.03 vs. 0.24 ± 0.01 µmol/g protein, respectively) as shown in Table 2.

Quantification of endothelial nitric oxide synthase (eNOS) expression in kidney

The expression of endothelial nitric oxide synthase (eNOS) in the cortex of the kidney resulted in significant 74% down regulation (p < 0.05) when compared to its expression in Control WKY which was taken as 100% (Control WKY vs. LVH-WKY: 1.0 ± 0.02 vs. 0.26 ± 0.02, respectively) as shown in Figure 1.

Table 3. The data showing the mean arterial pressure (MAP), pulse wave velocity (PWV) and renal cortical blood perfusion (RCBP) obtained on final day during the acute experiment of Control WKY and LVH-WKY. Data presented as the mean ± SEM. p < 0.05, * p < 0.05 vs. Control WKY.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control WKY</th>
<th>LVH-WKY</th>
</tr>
</thead>
<tbody>
<tr>
<td>MAP (mmHg)</td>
<td>104 ± 5</td>
<td>164 ± 15*</td>
</tr>
<tr>
<td>PWV (m/s)</td>
<td>6.2 ± 0.4</td>
<td>8.3 ± 0.4*</td>
</tr>
<tr>
<td>RCBP (bpu)</td>
<td>152 ± 10</td>
<td>99 ± 5*</td>
</tr>
</tbody>
</table>

bpu = blood perfusion unit.

Figure 2. Line graph shows dose dependent curves of the renal vasoconstrictors responses to the graded doses of adrenergic agonists (A) NA (B) PE and (C) ME in control WKY and LVH WKY in saline phase, CEC low dose phase and CEC high dose phase. 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 saline phase. *p < 0.05 vs. saline phase. # p < 0.05 vs. CEC low dose.
Measurement of systemic hemodynamics

Among systemic hemodynamics, mean arterial blood pressure was observed during the acute experiment while pulse wave velocity and renal cortical blood perfusion were the parameters of prime objective. The MAP was increased significantly (p < 0.05) in LVH-WKY (164 ± 15 mmHg) when compared to Control WKY (104 ± 5 mmHg). Pulse wave velocity was also increased significantly (p < 0.05) in LVH-WKY (8.3 ± 0.4 m/s) when compared to Control WKY (6.2 ± 0.4 m/s). Renal cortical blood perfusion was reduced significantly (p < 0.05) in LVH-WKY (152 ± 10) when compared to Control WKY (99 ± 5) as shown in Table 2.

Acute vasoconstrictor study

The adrenergic agonists NA, PE and ME produced dose dependent renal vasoconstrictions in both control WKY and LVH-WKY groups as shown in Figure 2 (A, B, C). The magnitude of the renal vasoconstrictor responses to NA, PE and ME in the saline phase in the LVH-WKY were significantly (p < 0.05) attenuated as compared to control WKY group (NA, 53 ± 3 vs. 28 ± 2%; PE, 49 ± 3 vs. 32 ±
1%; ME, 54 ± 1% vs. 33 ± 2%) when data were expressed as overall mean % drop in RCBP as shown in Figure 3 (A, B, C).

In the low dose of antagonist phase, the renal vasoconstriction responses to NA, PE and ME were attenuated in LVH group when the comparison was done against saline phase of Control WKY (NA: 52 ± 4 vs. 19 ± 1%; PE: 44 ± 3 vs. 30 ± 1%; ME: 45 ± 2 vs. 27 ± 1%) as shown in Figure 3 (A, B, C). Similarly in high dose phase CEC, the renal vasoconstriction responses to NA, PE and ME were attenuated in LVH group when the comparison was done against low dose phase CEC of control WKY (NA: 42 ± 5 vs. 23 ± 2%; PE: 44 ± 3 vs. 24 ± 1% and ME: 34 ± 2 vs. 23 ± 1%) as shown in Figure 3 (A, B, C). When responses of NA, PE and ME were compared with the saline phase, low dose CEC and high dose CEC of Control WKY with LVH-WKY, there was significant attenuation in the responses of all adrenergic agonists (NA, PE and ME) in LVH-WKY in all three phases with respect to corresponding phase of Control WKY as shown in Figure 3 (A, B and C).

DISCUSSION

Present study, perhaps for the first time, explored the functional contribution of α1B adrenoreceptor, chloroethylclonidine sensitive; modulating the renal vasoconstriction responses induced by adrenergic agonists in LVH induced WKY rats. In normal rats with no renal impairment, this functional contribution of α1B adrenoreceptor subtype was absent. Large body of evidences have shown in different pathological conditions with renal impairments these adrenergically induced renal vasoconstriction responses were either increased or decreased by chloroethylclonidine, especially in diabetes and diabetes combined with hypertension, heart failure and experimental hypertension (5, 29, 36). Present study was designed with the objective that induction of LVH would impart the functional contribution to α1B adrenoreceptor in the renal vasculature and identify the factors which are responsible for this renal impairment in LVH.

Elevated ACE activity in the plasma in present study represents elevated status of angiotensin II and this elevated levels of angiotensin II is in line with previous studies (37, 38) in LVH. In present study, ACE activity was not measured in kidney tissue however, the plasma level depict that similar kind of situation pertained in the kidney as well. Elevated level of angiotensin II is accused for many pathological states like oxidative stress by activating NADPH oxidase (39) and arterial stiffness (40). The above notion that angiotensin II increased oxidative stress can be supported by the increased plasma and kidney tissue oxidative level markers like superoxide dismutase (SOD), malondialdehyde (MDA) and glutathione (GSH) in LVH-WKY in present study when compared to Control WKY. It can be speculated that increased oxidative stress in kidney tissue indicates the increased ACE activity in the kidney along within the plasma. Increased oxidative stress can be considered as one of the contributing factors for renal impairment in LVH, which is in agreement with early reported data that state that oxidative stress is responsible for renal impairment (41). Increased ACE activity can be supported with the contention that renal cortical blood perfusion (RCBP) is decreased in LVH-WKY, which might be due to increased local vasoconstriction in the kidney due to elevated angiotensin II, noradrenaline (42, 43) and oxidative stress, which lead to vasoconstriction (41). It can be deduced from the arguments that increased ACE activity leads to increased oxidative stress in the plasma and kidney along with decrease in RCBP.

In present study, we observed NO deficiency in the plasma and kidney tissue of LVH-WKY. This decreased NO in the plasma is in line with few early studies which explored NO deficiency in LVH (44, 45). This deficiency of NO in systemic as well as in kidney tissue unfolded the unexplained reasons for renal impairment in LVH as NO is known as kidney regulator (41). One of the reasons might be the increased oxidative stress in the kidney as increased ROS production reduced the active NO (46). The decreased concentration of NO in plasma and kidney indicates the increased oxidative stress in the kidney and systemic circulation of LVH rats. This decreased NO in plasma and kidney can be accused for the increased arterial stiffness, which is evident in present study. The LVH-WKY group have high arterial stiffness as compared to Control WKY, which is in line with previous reports that NO is responsible for regulation of arterial stiffness (47, 48). In this model of LVH, angiotensin II level is also elevated, which is associated with an imbalance in NO/cGMP pathway leading to endothelial dysfunction (49). Another reason for reduced NO in the plasma and in the kidney may be down regulation of endothelial nitric oxide synthase (eNOS), NO is synthesized by the enzyme eNOS (21) and is known as a potent vasodilator (22). The decreased concentration of NO was justified by the down regulation of eNOS mRNA in the cortex of the kidney in present study. This down regulation of eNOS/NO
pathway in the kidney apparently indicates the down regulation of vasodilator pathway in the kidney. The eNOS/NO pathways generate vasodilation, and share guanylyl cyclase/guanosine monophosphate pathway which is operated by a G-protein coupled second messenger pathway system. The adrenergic receptors also operate via a G-protein coupled pathway (19) so down regulation of the counter regulatory eNOS/NO pathways in the kidney may also down regulate $\alpha_1$ adrenergic receptors in the kidney.

Administration of NA and PE in Control WKY did not influence the renal vasoconstriction in CEC low dose and CEC high dose phase, which indicates that $\alpha_{1B}$ adrenergic receptor is not functionally mediating the renal artery vasoconstriction. These findings support the previous studies (5, 29, 36) which stated that in normal rats with no renal impairment, there is no functional contribution of $\alpha_{1B}$ adrenergic receptor in mediating the adrenergically induced renal vasoconstriction. However, administration of ME in Control WKY resulted in blunted responses in CEC low dose and CEC high dose phases in Control WKY. This non responsiveness to NA and PE while blunted responses to ME indicates the functional shift of adrenergic receptors and renal vasoconstriction in normal WKY may be mediated by either $\alpha_{1A}$ or $\alpha_{1D}$ adrenergic receptors. It is reported that $\alpha_{1A}$ adrenergic receptors are functional subtypes in renal vasculature of normal rats (50) while functional contribution of $\alpha_{1D}$ adrenergic receptors in Control WKY and LVH-WKY is also reported (6). This blunted responses to ME in Control WKY may be due to affinity of ME to $\alpha_{1B}$ adrenergic receptors as it is reported that $\alpha_{1B}$ and $\alpha_{1A}$ exhibit similar kind of affinity for few ligands used for receptors characterization (17). So it can be assumed that ME has greater affinity for $\alpha_{1A}$ (51) and when compared to $\alpha_{1D}$ (52) and ME has affinity for $\alpha_{1D}$, adrenergic receptors in the Control WKY. Our study is in line with previous study which claimed low contribution of $\alpha_{1B}$ adrenergic receptors in SHR by using agonists and renal nerve stimulation (53). On the other hand, our findings are also in line with previous report (54) that CEC can block $\alpha_{1D}$ adrenergic receptors and then $\alpha_{1A}$ adrenergic receptors mediate the renal vasoconstriction.

Interestingly, when responses of NA, PE and ME were examined under the CEC low dose and CEC high dose there were blunted responses by $\alpha_{1B}$ adrenergic receptors to these adrenergic agonists indicating the functional importance of this adrenergic receptor subtypes in LVH. Our study is in line with few other studies which state that $\alpha_{1B}$ adrenergic receptors appeared to play a greater role in combined state of renal failure and hypertension (53) and high sodium salt intake (55). When responses to NA, PE and ME in saline, CEC low dose and CEC high dose phases of LVH-WKY on $\alpha_{1B}$ adrenergic receptors were compared with same phases of Control WKY, there was attenuation of responses to NA, PE and ME in all three phases of LVH-WKY. It was noteworthy to know that the magnitude of % drop in RCBP was reduced in LVH-WKY when compared to Control WKY. These findings suggested that $\alpha_{1B}$ adrenergic receptors are the functional subtype in mediating vasoconstriction in the renal vasculature of LVH-WKY.

**CONCLUSION**

It can be summarized that in Control WKY functional contribution is not mediated by $\alpha_{1B}$ adrenergic receptors but induction of LVH attributed $\alpha_{1B}$ adrenergic receptors as functional subtype mediating the renal vasoconstriction. However, in LVH-WKY there was a decrease in % drop in renal cortical blood perfusion and attenuation in vasoconstriction responses of $\alpha_{1B}$ adrenergic receptors to adrenergic agonists. This decrease in vasoconstriction responses of $\alpha_{1B}$ adrenergic receptors may be attributed to down regulation of eNOS/NO pathway in the kidney and increased oxidative stress in the plasma and kidney.

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