CORA Cork Open Research Archive
Cartlann Taighde Oscailte Chorcaí

Title	Interaction between nitric oxide and renal α1-adrenoreceptors mediated vasoconstriction in rats with left ventricular hypertrophyin Wistar Kyoto rats
Authors	Ahmad, Ashfaq;Sattar, Munavvar A.;Azam, Maleeha;Khan, Safia A.;Bhatt, Owais;Johns, Edward J.
Publication date	2018
Original Citation	Ahmad, A., Sattar, M. A., Azam, M., Khan, S. A., Bhatt, O. and Johns, E. J. (2018) 'Interaction between nitric oxide and renal α1-adrenoreceptors mediated vasoconstriction in rats with left ventricular hypertrophyin Wistar Kyoto rats', PLOS ONE, 13(2), e0189386 (21pp). doi: 10.1371/journal.pone.0189386
Type of publication	Article (peer-reviewed)
Link to publisher's version	http://journals.plos.org/plosone/article?id=10.1371/journal.pone.0189386 - 10.1371/journal.pone.0189386
Rights	© 2018, Ahmad et al. This is an open access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited. - https://creativecommons.org/licenses/by/4.0/
Download date	2024-04-19 12:18:31
Item downloaded from	https://hdl.handle.net/10468/5937

UCC
University College Cork, Ireland
Coláiste na hOllscoile Corcaigh

PLOS ONE

RESEARCH ARTICLE

Interaction between nitric oxide and renal α_1-adrenoreceptors mediated vasoconstriction in rats with left ventricular hypertrophyin *Wistar Kyoto* rats

Ashfaq Ahmad[1,2]*, Munavvar A. Sattar[1], Maleeha Azam[3], Safia A. Khan[1], Owais Bhatt[2], Edward J. Johns[4]

1 School of Pharmaceutical Sciences, Universiti Sains Malaysia, Penang, Malaysia, **2** Department of Pharmacology and Toxicology, Virginia Commonwealth University, Richmond, Virginia, United States of America, **3** Translational Genomics Lab, Department of Biosciences, COMSATS Institute of Information Technology, Islamabad, Pakistan, **4** Department of Physiology, University College Cork, Cork, Ireland

* aahmad2@vcu.edu

Check for updates

OPEN ACCESS

Citation: Ahmad A, Sattar MA, Azam M, Khan SA, Bhatt O, Johns EJ (2018) Interaction between nitric oxide and renal α_1-adrenoreceptors mediated vasoconstriction in rats with left ventricular hypertrophyin *Wistar Kyoto* rats. PLoS ONE 13(2): e0189386. https://doi.org/10.1371/journal.pone.0189386

Editor: Michael Bader, Max Delbruck Centrum fur Molekulare Medizin Berlin Buch, GERMANY

Received: July 3, 2017

Accepted: November 26, 2017

Published: February 15, 2018

Copyright: © 2018 Ahmad et al. This is an open access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Data Availability Statement: All relevant data are within the paper and its Supporting Information files.

Funding: The Institute of Postgraduate Studies (IPS) is acknowledged for the provision of a USM fellowship (Teaching) to Ashfaq Ahmad (APEX (1002/JHEA/ATSG4001)). The authors fully acknowledge USM-RU grant no. 1001/PFARMASI/815078 for this work to Munavvar A. Sattar.

Abstract

Left ventricular hypertrophy (LVH) is associated with decreased responsiveness of renal α_1-adrenoreceptors subtypes to adrenergic agonists. Nitric oxide donors are known to have antihypertrophic effects however their impact on responsiveness of renal α_1-adrenoreceptors subtypes is unknown. This study investigated the impact of nitric oxide (NO) and its potential interaction with the responsiveness of renal α_1-adrenoreceptors subtypes to adrenergic stimulation in rats with left ventricular hypertrophy (LVH). This study also explored the impact of NO donor on CSE expression in normal and LVH kidney. LVH was induced using isoprenaline and caffeine in drinking water for 2 weeks while NO donor (L-arginine, 1.25g/Lin drinking water) was given for 5 weeks. Intrarenal noradrenaline, phenylephrine and methoxamine responses were determined in the absence and presence of selective α_1-adrenoceptor antagonists, 5- methylurapidil (5-MeU), chloroethylclonidine (CeC) and BMY 7378. Renal cortical endothelial nitric oxide synthase mRNA was upregulated 7 fold while that of cystathione γ lyase was unaltered in the NO treated LVH rats (LVH-NO) group compared to LVH group. The responsiveness of renal α_{1A}, α_{1B} and α_{1D}-adrenoceptors in the low dose and high dose phases of 5-MeU, CEC and BMY7378 to adrenergic agonists was increased along with cGMP in the kidney of LVH-NO group. These findings suggest that exogenous NO precursor up-regulated the renal eNOS/NO/cGMP pathway in LVH rats and resulted in augmented α_{1A}, α_{1B} and α_{1D} adrenoreceptors responsiveness to the adrenergic agonists. There is a positive interaction between H_2S and NO production in normal animals but this interaction appears absent in LVH animals.

Competing interests: The authors have declared that no competing interests exist.

Introduction

Left ventricular hypertrophy (LVH) is characterized by overstimulation of the heart due to hyperactivity of the sympathetic nervous system and both circulating noradrenaline and mean discharge frequency in peripheral sympathetic nerves have been reported elevated in hypertensive LVH patients [1].At an experimental level, renal sympathetic nerve activity was found to be elevated in rats with essential hypertension and LVH compared to the control *Wistar Kyoto* rats [2]. This sympatho-activation is associated with vascular dysfunction and impairment of α_1-adrenoceptor-mediated renal vasoconstriction [3].This attenuation of α_1-adrenoceptor-mediated renal vasoconstrictor responsiveness to adrenergic agonists in states of hypertension and renal failure has been studied previously [4]. Moreover, a decrease in responsiveness of α_{1D}–adrenoreceptors to adrenergic agonists when administered exogenously has been reported LVH [5]. However, the question of the role of NO on the responsiveness of α_1-adrenoceptors in LVH remains unanswered.

Higher levels of noradrenaline (NA) and angiotensin II (Ang II) in the plasma have been found in rat models of LVH induced using isoprenaline and caffeine [5–7] At the level of renal vasculature, catecholamines are released at the sympathetic nerve neuro-effector junctions and activate the G-protein operated adrenoreceptors which increase cytosolic Ca^{2+} concentration to vascular smooth muscle contractions [8]. Pharmacological and cloning studies have reported three subtypes of α_1-adrenoceptors, α_{1A}, α_{1B} and α_{1D} [9]. These α_1-adrenoceptors are operated by G-protein coupled receptor 2^{nd} messenger signalling pathway [9]. Increased vasoconstriction due to elevated NA and Ang II can be suppressed as a result of an up-regulation of the NO-cGMP pathway which is responsible for inhibition of L-type Ca^{2+} channels [10] which induce a vasodilation.

Nitric oxide derived from endothelial nitric oxide synthase (eNOS) is important in maintaining and determining normal renal hemodynamic and tubular reabsorptive function [11, 12]. Nitric oxide has been reported to reduce renal ischemia reperfusion injury [13] both directly and indirectly [14].There is evidence demonstrating that NO exerts a tonic role in the medullary circulation [15] where it seems to have a higher concentration than in the cortex [16]. Earlier studies have shown that intravenous infusion of endothelial cells (eNOS) in ischemic kidney provides dramatic renoprotection by lowering plasma creatinine [17, 18]. We reported recently that the down regulation of the eNOS/NO pathway was associated with a decrease in responsiveness of α_{1A}–adrenoreceptors to adrenergic agonists in the kidney of LVH rats [19]. Decreased responsiveness of α_1-adrenoreceptors has been reported in many pathological conditions such as hypertension and renal failure [4], in fructose fed rats and in LVH [5]. Although these studies provide an elegant insight as to the renal consequences of reduced responsiveness of α_1-adrenoceptors to adrenergic agonists, no study has been conducted to determine the impact of an increase in the responsiveness of α_1-adrenoreceptors in different pathological conditions.

Various studies have shown that production of both H_2S and NO are interdependent [20–24] in regulating vascular tone. Literature showed that H_2S yield NO production in smooth muscles [25,26] while it has also been reported that NO enhanced the up regulation of H_2S production as reflected by plasma concentrations [27, 28].

The potential interaction between NO and α_1–adrenoceptor subtypes of normal or LVH animals in regulating renal hemodynamic has not been investigated to date. Collectively, the evidence available regarding NO plus our recent findings of an interaction between eNOS/NO α_1–adrenorecptors subtypes in the kidney of LVH rats raises a number of questions. The hypothesis to be explored is as follows: firstly, that upregulation of the eNOS/NO/cGMP pathway will increase the responsiveness of the renal vascular α_1–adrenoreceptor subtypes to

adrenergic agonists in LVH rats; secondly, that up regulation of eNOS/NO in kidney will improve renal cortical blood perfusion in LVH; thirdly, that chronic administration of L-arginine (an NO donor) will suppress the CSE/H_2S pathway in the kidney of LVH rats.

Materials and methods

Animals and induction of LVH

All the procedures of current study were approved by the Animal Research and Service Centre (ARASC) USM with approval no./2012/ (76) (364) and all the methods were performed by the guidelines and procedures as approved by ARASC. 84 male Wistar *Kyoto* (WKY) rats (200 ±10g) were recruited from the animal house of Universiti Sains Malaysia and kept in standard animal facility provided by School of Pharmaceutical Sciences, USM with free access to food and water. All animals were divided into three main groups; one for renal functional studies, a second group for CSE and eNOS mRNA evaluation and a third group for the measurement of nitric oxide synthase (NOS) protein expression. The main group for renal hemodynamic functional examination of α_1-adrenoceptors subtypes consisted of 12 subgroups groups. These groups were named according to antagonists used in that group. Renal functional study group consists of:

(1) Control-5MeU; (2) Control-CEC; (3) Control-BMY. LVH groups consisted of: (4) LVH-5MeU; (5) LVH-CEC; (6) LVH-BMY. Control groups treated with NO consisted of: (7) Control-NO+5MeU; (8) Control-NO+ CEC; (9) Control-NO+BMY.LVH groups treated with NO consisted of: (10) LVH-NO+5MeU: (11) LVH-NO+CEC; (12) LVH-NO+BMY (n = 6). Similarly, molecular study for quantification of CSE and eNOS mRNA expression consisted of 4 groups: Control, LVH, Control-NO and LVH-NO whereby the cortex part of left kidneys were taken for quantification of CSE and eNOS mRNAs expression. A third group, Control (Control-L-NIO) and a LVH group (LVH-L-NIO),which received L-N5-(1-iminoethyl)-ornithine), (10mg/kg I.P.) 15 minutes before the acute experiment [29] and NOS activity was compared to a control group (control-L-NIO).

LVH was induced by a modification of an earlier model [30] using isoprenaline (5mg/kg s.c) and caffeine as recently reported [5].Control-NO and LVH-NO group rats received L-arginine (1.25 g/L in the drinking water) was used as a donor of NO for 5 weeks as reported previously [31]. Control rats received i.ps injection of 0.9% NaCl.

Molecular expression of *CSE* and *eNOS mRNAs* in the cortex of the kidney

Molecular expression study was performed following the procedure reported earlier [19]. Conversion of RNA to cDNA was performed by using a High Capacity RNA-to-cDNA kit (Applied Biosystems™, USA) according to the manufacturer's instruction.

Different TaqMan primers and probes were used for gene which have following accession numbers; CSE gene (Gen Bank accession No. NM_017074.1 and Rn00567128_m1) [32]; eNOS genes (Gen Bank accession No. NM_021838.2 and Rn02132634_s1) [33, 34] and for the β-actingene (Gen Bank accession No. NM_031144.2 and Rn00667869_m1) were derived from TaqMan®-Gene Expression assays (Applied Biosystems, USA) [35, 36].TaqMan® Gene Expression assays were obtained and the procedure was followed according to the instructions of the manufacturer (Applied Biosystems™, USA).

Quantitative RT-PCR reactions were carried out on cortex of the left kidney. Amplification of the housekeeping enzyme (internal control) Beta actin allowed sample loading and normalization to be determined. The relative quantification of the target genes CSE, eNOS and internal control beta actin used the comparative C_T (threshold cycle) method with arithmetic formula ($2^{-\Delta\Delta CT}$) [37].

NOS enzyme activity in kidney

NOS enzyme activity was done as reported in earlier studies [38, 39]. Enzyme activity was expressed as citrulline production in femtomol per milligram of protein per minute.

Measurement of nitric oxide concentration in the plasma and kidney

The plasma and tissue concentration of nitric oxide was measured using kits as directed by manufacturer (NJJC Bio Inc., Nanjing, China) while protein quantity was measured using an early reported method [39, 40]. Blood was collected from the rat and centrifuged at 5000g for 10 minutes to collect plasma for analysis of nitric oxide.

Measurement of hydrogen sulphide concentration in the plasma

The plasma concentration of H_2S was measured as reported previously [41, 42].

Measurement of cGMP levels in the kidney

The method used followed the instructions provided by manufacture of the cGMP Direct Immunoassay Kit (Abcam). However, procedure involves sample preparation, construction of standard curve, followed by acylation and then quantification of cGMP by measuring the optical density at 450nm.

Acute experiment for renal vasoconstrictor responses

In vivo renal vasoconstrictor responses studies were performed as previously reported [43]. Animals were anaesthetized by intraperitoneal pentobarbitone sodium (60mg/kg, Nembutal®, CEVA, France) injections. Tracheotomy was done by inserting tubing in the trachea to facilitate breathing followed by cannulation of jugular vein and carotid artery for vehicle infusion and continuous MAP monitoring respectively. Furthermore, carotid artery cannula was connected to a pressure transducer (model P23 ID Gould, Statham Instruments, UK) which was further attached to a PowerLab data acquisition system (PowerLab, ADInstruments, Australia). A mid-line abdominal incision was made to expose the aorta and left kidney and a laser Doppler probe (ADInstruments, Australia) was placed on the cortical surface of the left kidney to measure renal cortical blood perfusion (RCBP). In order to facilitate the close infusion of adrenergic agonist noradrenaline (NA), phenylephrine (PE) and methoxamine (ME) close to the face of renal artery, left iliac artery was cannulated and cannula was pushed at the required level designed by study [44, 45]. Animals were allowed to stabilize for 1 hour before commencing acute vasoconstrictor studies.

Renal vasoconstrictor responses

Different doses of NA, PE and ME were administered intrarenally in ascending and descending order as described below; NA at 25, 50, 100 and 200ng; PE at 0.25, 0.5,1 and 2μg; ME at 1, 2, 3 and 4 μg. These drugs were prepared in saline (0.9g of NaCl/L of water) freshly every day and stored in 4˚C [42, 46]. A wash out time of 10 min was given to each dose administered to ensure washout of agonists [47, 48]. Overall acute experiment consisted of three phases, a saline or non-drug phase, a low dose antagonist phase and a high dose antagonist phase. In the saline phase, saline was infused intrarenally at a rate of 6ml/kg/h during which adrenergic agonists were infused in ascending and descending order. In the low and high dose phases, 5-MeU was administered close to renal artery at bolus dose of 5μg/kg and plus infusion of 1/4^{th} of the bolus dose as a continuous infusion (1.5μg/kg/h) to study α_{1A}adrenoreceptors while during the high dose phase 5-MeU was administered as a 10μg/kg bolus dose followed by a

continuous infusion of 2.5μg/kg/h. Chloroethylclonidine was administered in low dose (5mg/kg as bolus dose) and high dose (10 mg/kg as bolus) in kidney [49]. BMY 7378 was infused intrarenally at 100 and 200mg/kg plus 1/4th the dose as a continuous infusion, for the low and high dose phases, respectively, during which adrenergic agonists were administered [4].

Histopathology of kidney tissues using haematoxylin and eosin staining

At the end of experiment right kidneys were removed and tissues for all four groups were subjected to the histopathological process of staining as reported [39, 50].

Histopathology study of the kidney using picrosirus red stain kit

The same preparative procedure given above was repeated for staining with Picrosirus red (Polyscience, Inc. Germany) as reported [39] and directed by manufacturer.

Preparation of agonists and antagonists

5-methylurapidil (RBI, Natick, MA, USA) is a selective blocker of α_{1A}adrenoreceptors [51], chloroethylclonidine (RBI, Natick, MA, USA) a selective blocker of α_{1B}adrenoreceptors [52] and BMY 7378 (8-(2-(4-(2-methoxyphenyl)-1-piperazinyl)ethyl)-8-azaspiro(4,5) decane-7,9-dione dihydrochloride; RBI) a selective blocker of α_{1D} adrenoreceptors [53], were prepared in saline and kept frozen as stock solutions.

Statistical analysis

The renal vasoconstrictor response to each agonist was taken as the mean of ascending and descending responses due to four doses which are shown as line graphs as shown in supplementary data. The comparison between the groups considered the overall response calculated as the mean of the % of drop in renal cortical blood profusion pressure. All data was presented as mean ± S.E.M. The renal vasoconstrictor data were subjected to a one-way ANOVA followed by a Bonferroni *post hoc* test using GraphPad Prism (GraphPad SoftwareInc., CA, USA) with significance taken at $P< 0.05$. The gene expression data was analysed using the comparative method ($\Delta\Delta C_T$ method) and using the StepOne™ Software (Version 2.1, Applied Biosystem, USA).

Results

Molecular expression of renal cortical *CSE* and *eNOS*

Induction of LVH resulted in a 79% down regulation of eNOS mRNA in the renal cortex compared to that in the control rats. Treatment of LVH with L-arginine resulted in the 510% increase in eNOS mRNA when compared to LVH groups as shown in Fig 1A

Induction of LVH resulted in a 73% down regulation of CSE mRNA in the renal cortex compared to its expression in the control rats. Treatment with L-arginine in the Control rats increased CSE mRNA by 204% compared to the untreated counterpart but had no impact on the expression levels in the LVH rats as shown in Fig 1B.

NOS enzyme activity in kidney

Ca^{2+}-dependent NOS activity was reduced significantly (all $P<0.05$) in the kidney of LVH when compared to the control group while exogenous administration of L-arginine in LVH significantly increased (all $P<0.05$) NOS activity when compared to LVH as shown in Fig 1C.

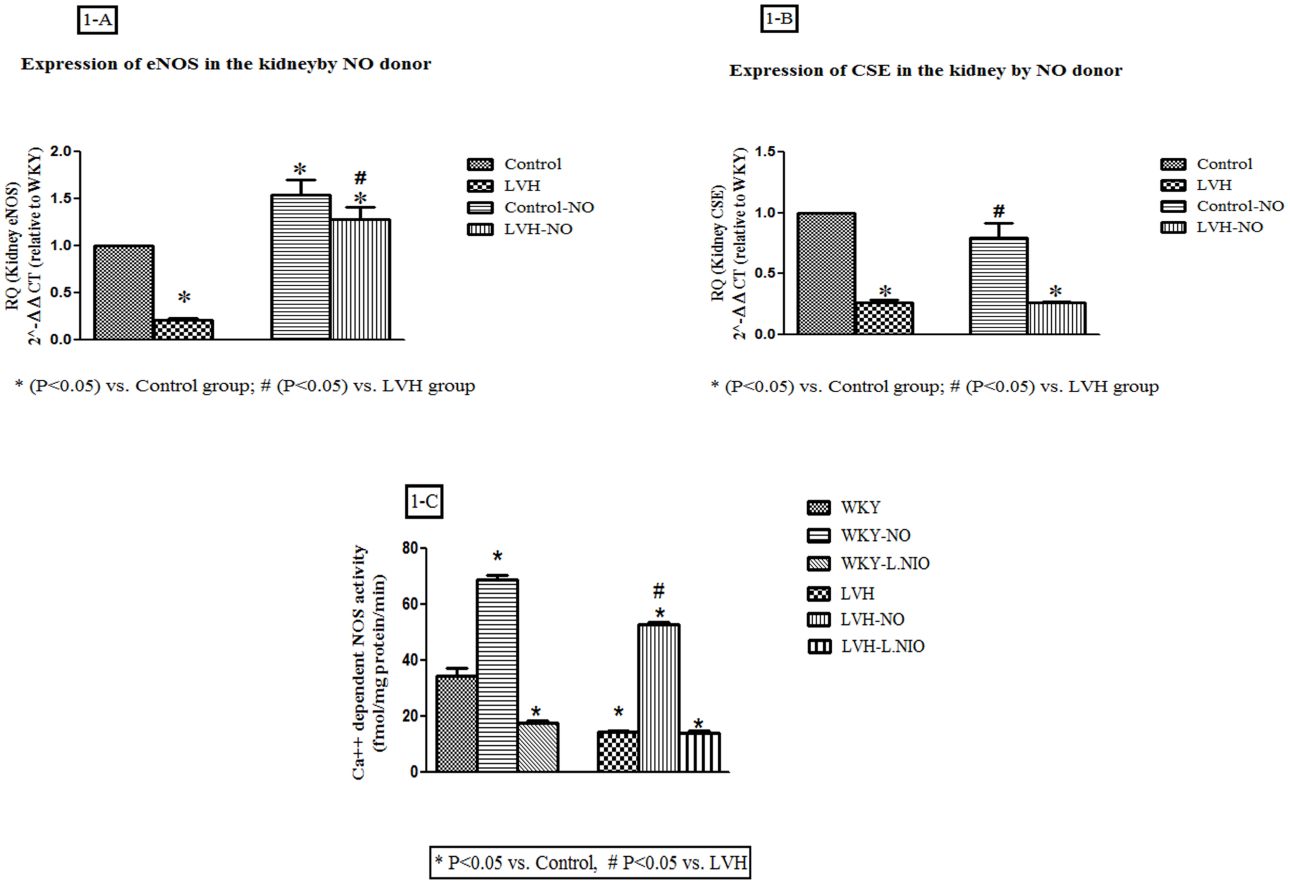


Fig 1. A, B, C) showing the molecular expression of CSE and eNOS mRNAs and NOS activity in the cortex of kidney of the Control, LVH, Control-NO and LVH-NO rats. Data is shown as ± SEM while significance is taken as $p<0.05$.

https://doi.org/10.1371/journal.pone.0189386.g001

Renal and plasma nitric oxide concentrations

Induction of LVH resulted in 45% decrease in renal NO concentrations compared to the control rats. Treatment with L-arginine resulted in a 236% and 173% increase in renal NO concentrations in the Control-NO and LVH-NO groups, respectively as shown in Fig 2A.

Induction of LVH caused a 29% decrease in plasma NO concentration compared to that in the control rats. Treatment with L-arginine resulted in increased plasma NO concentration of 71% and 52% in the Control-NO and LVH-NO groups, respectively as shown in Fig 2B.

Plasma hydrogen sulphide concentrations

The plasma concentration of H_2S was significantly ($P<0.05$) lower in the LVH group compared to the Control group (16±1 vs.37±1μM) and was unchanged following treatment with L-arginine compared to LVH (18± vs. 16±1μM) as shown in Fig 2C.

Renal cGMP concentrations

Induction of LVH decreased renal concentrations of cGMP by 84% compared to the control rats. Treatment with L-arginine increased renal cGMP concentrations by 216% and 163% in the Control-NO and LVH-NO groups, respectively as shown in Fig 2D.

Renal cortical blood perfusion

RCBP was 46% lower (P<0.05) in the LVH compared to the Control rats. Treatment with L-arginine resulted in a higher RCBP in Control-NO and LVH-NO of some 36% and 47%, respectively as shown in Fig 2E.

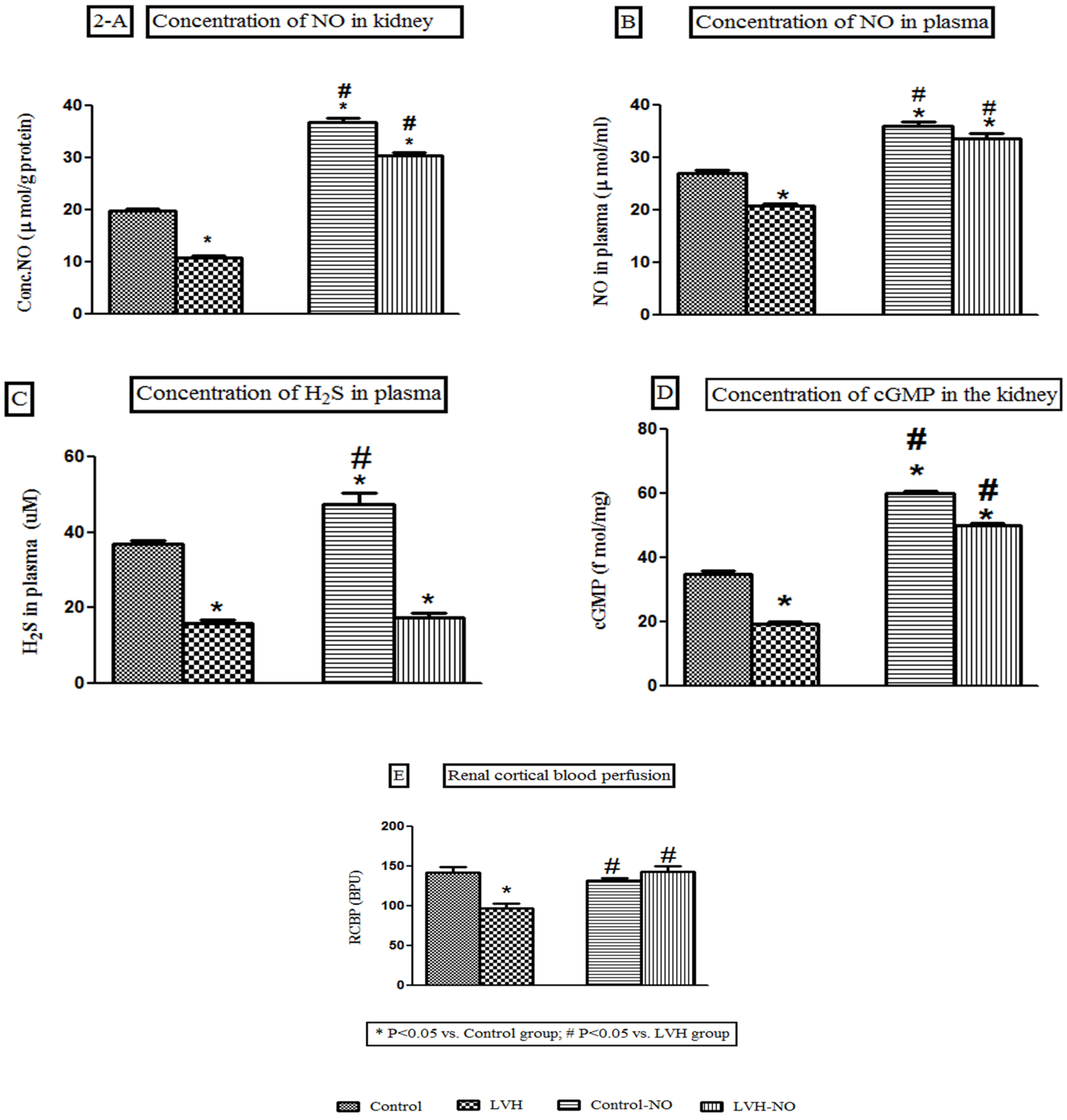


Fig 2. Showing the concentration of nitric oxide in the kidney (A), nitric oxide in plasma(B), H_2S in plasma (C), cGMP in the kidney (D) and renal cortical blood perfusion of Control, LVH, Control-NO and LVH-NO rats. Data is shown as ± SEM while significance is taken as p<0.05. * (P<0.05) vs. Control group; # (P<0.05) vs. LVH group.

https://doi.org/10.1371/journal.pone.0189386.g002

Renal vasoconstrictor responses of α_{1A}–adrenorecptors to adrenergic agonists

Noradrenaline. The reductions in renal cortical blood perfusion (RCBP) in the LVH group were 33% lower in the saline and 37% during the high dose phases of the antagonists ($P<0.05$) when compared to same phases in the Control groups of rats. Treatment of LVH with L-arginine resulted in augmented responses to the α_{1A}–adrenoreceptor agonist NA, by 93% in the saline phase, 76% in low dose 5-MeU phase and 158% in the high dose 5-MeU phase when compared to respective phases in the LVH group as shown in Fig 3A (S1 Fig).

Phenylephrine. There was a reduced responsiveness to PE in the LVH group, of 27% in saline and 46% in the high dose phase of 5-MeU ($P<0.05$) while there was no significant difference observed during the low dose phase of antagonist when compared to same phases of Control groups of rats. Treatment of LVH with L-arginine augmented the RCBP responses to PE by 97% in the saline and 123% in high dose 5-MeU phases when compared to respective phases of the LVH group as shown in Fig 3B (S2 Fig).

Methoxamine. There was a reduced RCBP responsiveness in the LVH group to ME by 29% in saline phase, 31% in low dose and 43% in high dose 5-MeU phases when compared to same phases of Control group of rats. Treatment of LVH with L-arginine resulted in augmented responses to ME by 110% in the saline, 91% in low dose phase and 217% in high dose 5-MeU phases ($P<0.05$) when compared to the respective phases in the LVH group as shown in Fig 3C (S3 Fig).

Renal vasoconstrictor responses of α_{1B}–adrenorecptors to adrenergic agonists

Nordrenaline. The responsiveness of RCBP to NA was lower by 47% in the saline, 63% in low dose and 52% in high dose CEC phases when compared to the same phases in the Control groups of rats. Treatment of LVH with L-arginine augmented the RCBP responses to NA by 75% in the saline, 116% in the low dose and 75% in the high dose CEC phase compared to respective phases of the LVH group as shown in Fig 4A (S4 Fig).

Phenylephrine. The RCBP responsiveness to PE was lower by 35% in the saline phase, 36% in low dose and 45% in high dose CEC phases compared to same phases in the Control groups of rats. Treatment of LVH with L-arginine resulted in augmented RCBP responses PE by 88% in the saline, 75% in low dose and 79% in the high dose CEC phases compared to the respective phases of in the LVH group as shown in Fig 4B (S5 Fig).

Methoxamine. There was a blunted RCBP responsiveness to ME by 36% in the saline, 40% in the low dose and 32% in high dose CEC phases when compared to the same phases in the Control groups of rats. Treatment of LVH with L-arginine resulted in augmented RCBP responses to ME, by 74% in the saline, 137% in low dose % in high dose CEC phases compared to the respective phases in the LVH group as shown in Fig 4C (S6 Fig).

Renal vasoconstrictor responses of α_{1D}–adrenorecptors to adrenergic agonists

Nordrenaline. In the LVH the RCBP responses to NA were lower by 22% in saline and 19% in high dose BMY phase compared to same phases of Control groups of rats. Treatment of the LVH group with L-arginine augmented the RCBP responses to NA by 65% in the saline, 50% in the low dose and 77% in the high dose BMY phases of when compared to respective phases of the LVH group as shown in Fig 5A (S7 Fig).

Phenylephrine. Following LVH induction, there were smaller RCBP responses to PE, by 23% in the saline and 33% in the high dose BMY phase compared to the same phases in the

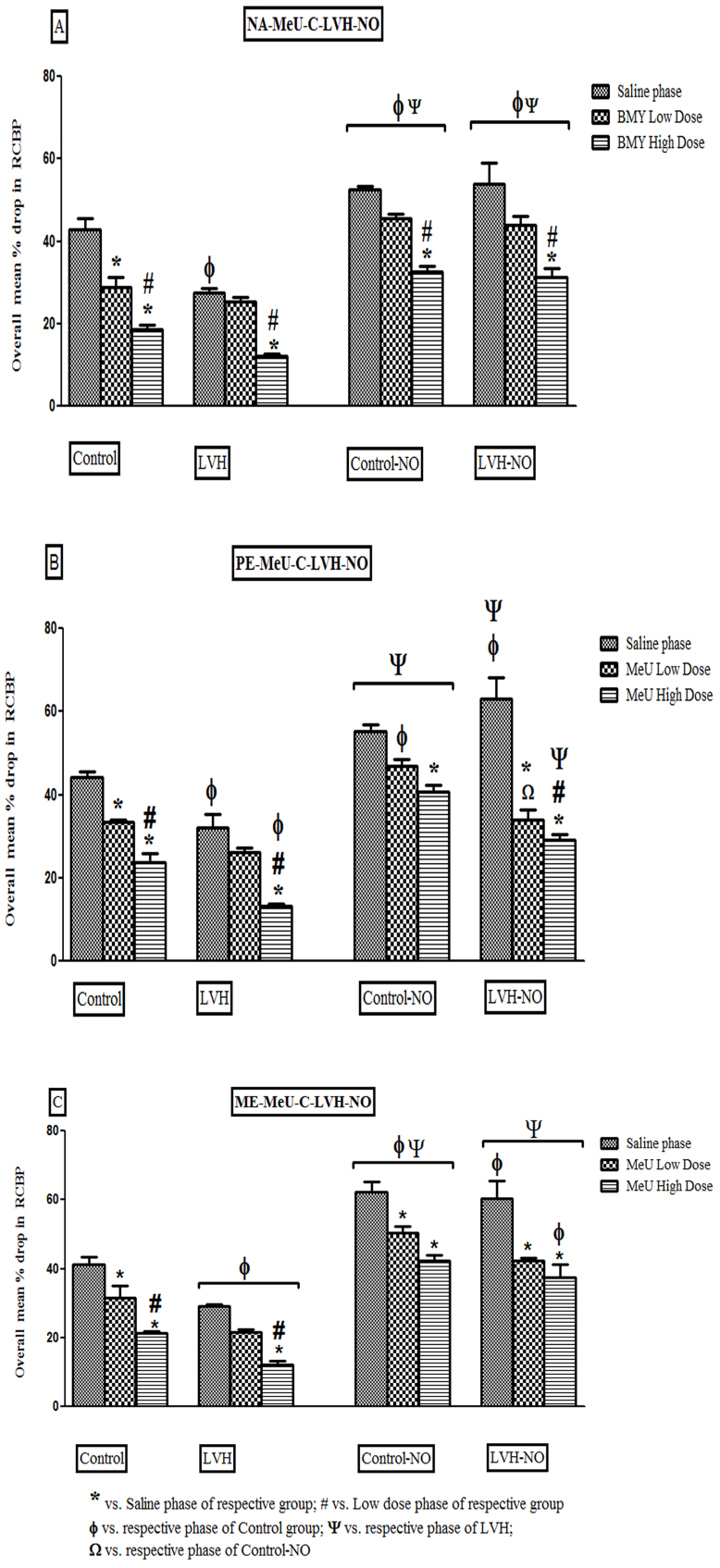

A
NA-MeU-C-LVH-NO
Overall mean % drop in RCBP
Saline phase
BMY Low Dose
BMY High Dose
Control
LVH
Control-NO
LVH-NO
B
PE-MeU-C-LVH-NO
Saline phase
MeU Low Dose
MeU High Dose
C
ME-MeU-C-LVH-NO
* vs. Saline phase of respective group; # vs. Low dose phase of respective group
ϕ vs. respective phase of Control group; Ψ vs. respective phase of LVH;
Ω vs. respective phase of Control-NO

Fig 3. Showing the renal vasoconstrictor responses of α_{1A}adrenoreceptors to NA (3A), PE (3B) and ME (3C) in the kidney of Control, LVH, Control-NO and LVH-NO rats. Data is shown as ± SEM while significance is taken as $p<0.05$.

https://doi.org/10.1371/journal.pone.0189386.g003

Control group of rats. Treatment of LVH with L-arginine augmented the RCBP responses to PE, by 83% in the saline phase, 104% in low dose BMY phase and131% in the high dose BMY phase compared to respective phases in the LVH group as shown in Fig 5B (S8 Fig).

Methoxamine. There was a reduced RCBP responsiveness in the LVH group to ME, by 26% in saline phase and 46% in high dose BMY phase compared to same phases of Control group of rats. Treatment of LVH with L-arginine resulted in augmented RCBP responses to ME, by 68% in the saline phase, 128% in low dose BMY phase and254% in the high dose BMY phase compared to the respective phases in the LVH group as shown in Fig 5C (S3 Fig).

Histopathological evidence

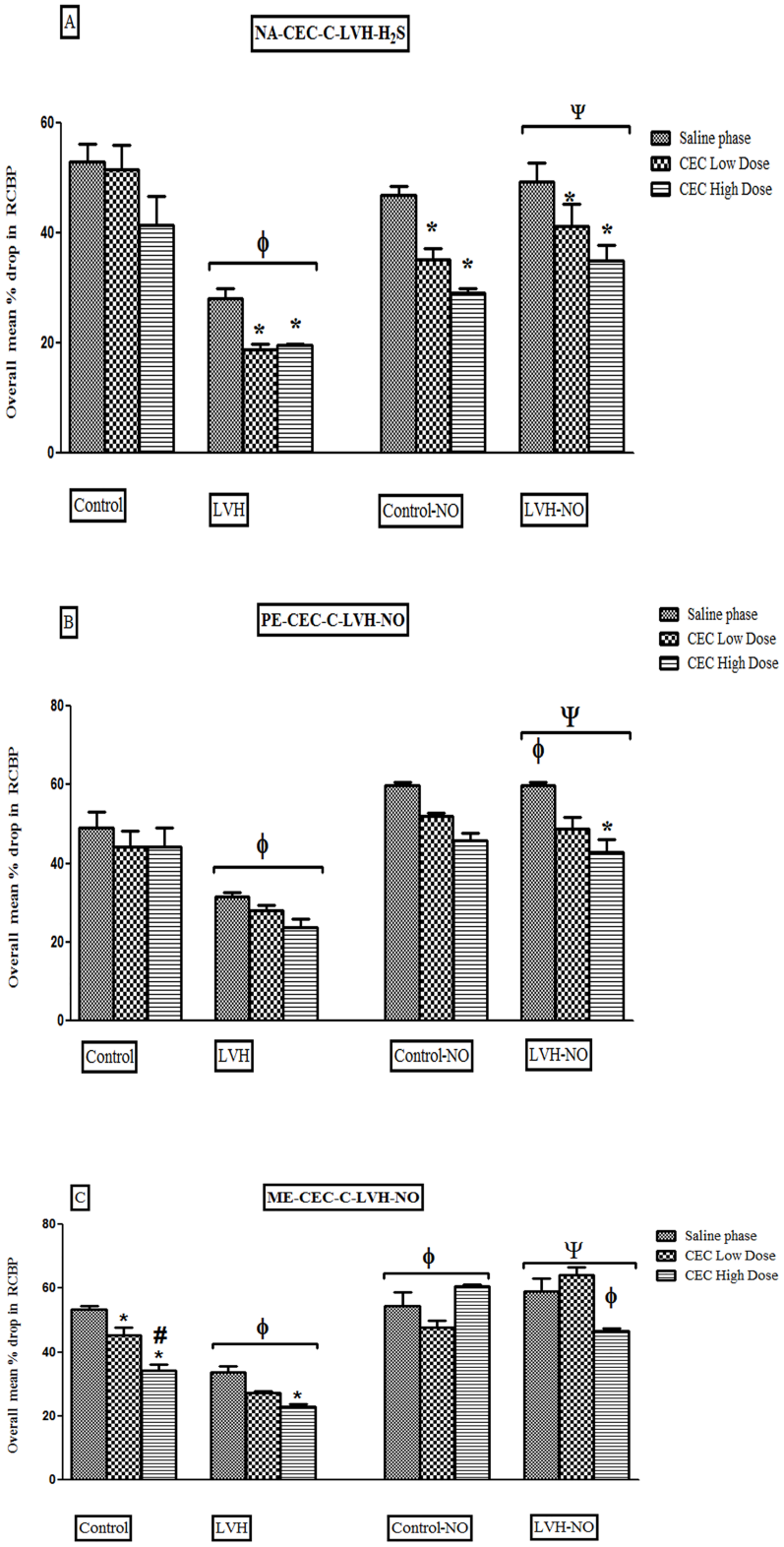
The kidney tissue did not show any ultra-structural changes in glomerular and tubular components in the LVH group except hypercellularity of the glomerulus with increased mesangial and endothelial cells as shown in Fig 6A and 6B. Treatment with L-arginine in the LVH group resulted in normal glomerular structures but with a mild atrophy of the tubules. Blood vessels and parenchyma were normal in LVH-NO as shown in Fig 6C and 6D).

The kidney tissues of all four groups were subjected to PicroSirius red staining and observed under the polarized light. The collagen content appeared as a red colour as shown in Fig 6E, 6F and 6H) and collagen in the kidney tissue was quantified by using collagen detection software (Image+Pro+Plus6.0, ipwin32, USA).Induction of LVH increased the collagen content, which appeared as plaques around the glomerulus when compared to Control-WKY, as shown in Fig 6E and 6F. However, treatment with L-arginine diffused the bands and collagen deposition was reduced to thin threads and appeared as a network around the glomerulus when compared to the LVH- group as shown in Fig 6G and 6H. Software quantification score showed collagen deposition in Control, LVH, Control-NO and LVH-NO as 0.6%, 3.8%, 1.4% and 1.6% respectively.

Discussion

The present study was designed to explore the effect of exogenous administration of an NO precursor (L-arginine) on the eNOS/NO/cGMP pathway in the kidney of normal and LVH rats and to investigate whether there was a negative modulatory effect on the CSE/H_2S pathway in the LVH rats. The major hypothesis was that upregulation of the renal eNOS/NO/cGMP pathway in the kidney would not only prevent the reduced responsiveness in renal cortical blood perfusion but would also tend to normalise the blunted RCBP responses of α_1-adrenoreceptorsto adrenergic agonists the LVH rats.

The findings clearly showed that there was a down regulation of eNOS mRNA in the cortex of the kidney of the rats given isoprenaline/caffeine to induce LVH and indeed, the NO concentration in the renal cortex was lower. A reduced concentration of NO due to decreased expression of eNOS has previously been reported in the pathological states of hypertension [54] and LVH [19]. There are a number of possible causes for the reduced NO concentration, including reduced NO elaboration from eNOS, increased oxidative inactivation of NO and increased production of vasoconstrictors like endothelin-1 and thromboxane A2 [55, 56]. The down regulation of eNOS/NO occurs at a time when there is increased production of the vasoconstrictors noradrenaline and angiotensin II [3, 6,9] in this model of LVH. Nitric oxide induced vasodilation is followed by an increase in cGMP produced by soluble guanylyl cyclase



* vs. Saline phase of respective group; # vs. Low dose phase of respective group

ϕ vs. respective phase of Control group; Ψ vs. respective phase of LVH

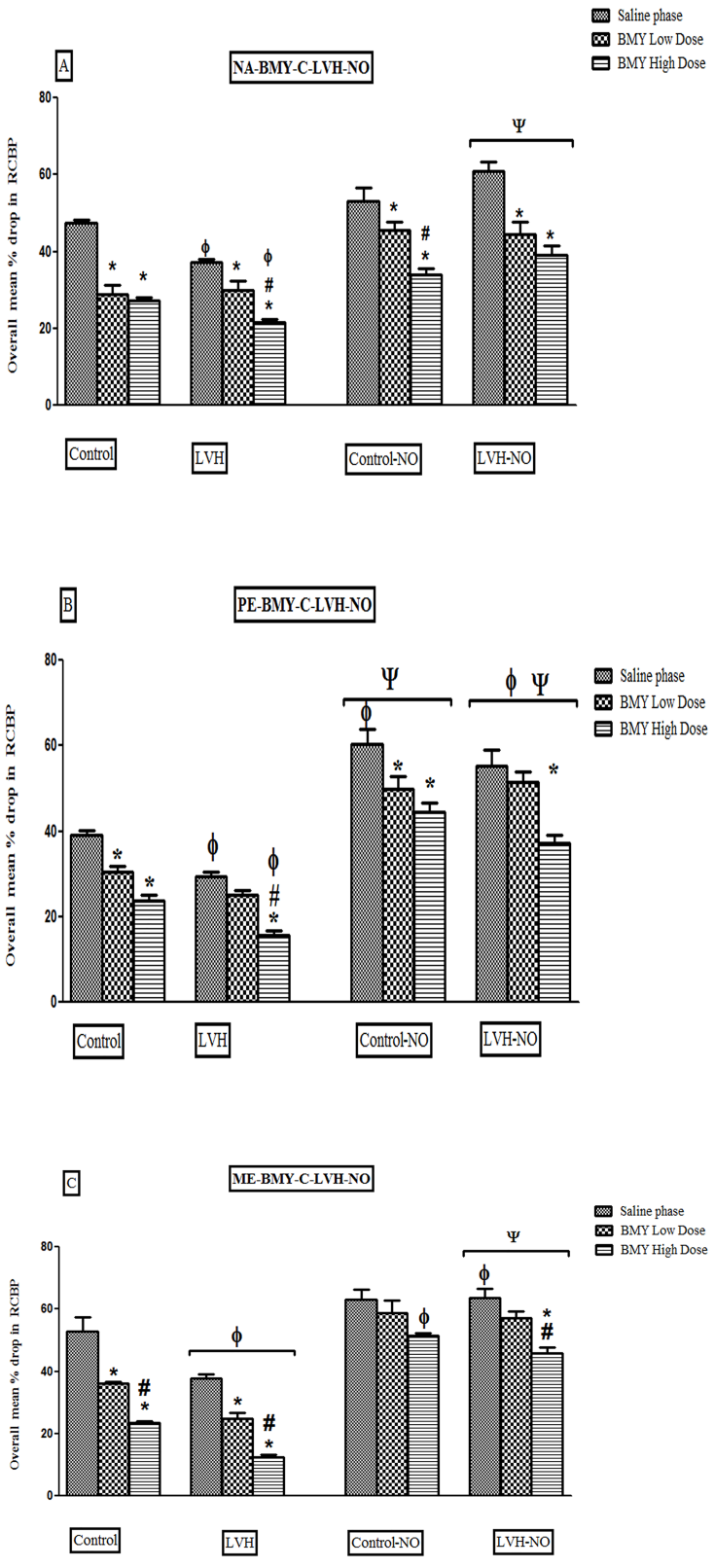
Fig 4. Showing the renal vasoconstrictor responses of α_{1B}adrenoreceptors to NA (4A), PE (4B) and ME (4C) in the kidney of Control, LVH, Control-NO and LVH-NO rats. Data is shown as ± SEM while significance is taken as $p<0.05$.

https://doi.org/10.1371/journal.pone.0189386.g004

[57] and in the present study this NO-cGMP axis is attenuated as found in other models of cardiac hypertrophy [58]. Although the down regulation of the eNOS/NO/cGMP was found in the kidney compartment, the fact that there was also reduced plasma NO concentration suggested that a similar situation pertained globally. This down regulation of the renal eNOS/NO/cGMP pathway in the LVH rats was associated with an increased renal vascular tone possibly due to the vasoconstrictor actions of noradrenaline and angiotensin II in the kidney. This would be supported by reports that Ang II suppressed NO-cGMP production [59] and thus elevated production of these vasoconstrictors might be responsible for the reduced basal renal cortical blood pressure in the present study. An attempt was made to provide further evidence for this view by up regulating the eNOS/NO/cGMP pathway using exogenous administration of L-arginine as an NO donor, with the aim of counteracting the effect produced by vasoconstrictors. It was evident that this approach resulted in an increased RCBP, as shown in Figs 2 and 3. Although previous studies have shown that NO increased papillary blood perfusion [15], the present study demonstrated that this also occurred in the renal cortex as renal cortical blood perfusion was increased at a time when the eNOS/NO/cGMP pathway was up regulated in the cortex of the kidney. This would suggest that the buffering action of NO would to a degree offset the actions of vasoconstrictors. Thus, the buffering action of NO to angiotensin II enhanced distensibility and increased eNOS/NO/cGMP pathway fit which would contribute to the increased RCBP.

The responsiveness of α_{1A}-adrenoreceptors to adrenergic agonists was attenuated, but not blocked, in the LVH model which indicated that the adrenergically mediated renal vasoconstriction was via α_{1A}-adrenoreceptors which are the predominant subtype in renal resistance vessels [60]. This decrease in α_{1A}-adrenoreceptor responsiveness may be related to compensatory mechanisms whereby the enhanced sympathetic nervous system activity leads to a down regulation or desensitization of receptors [61]. Sympathetic nervous activity is elevated in this model of LVH as reflected by the increased circulating levels of noradrenaline [3, 6]. It should be pointed out that an increased renal sympathetic nerve activity will also stimulate renin release and hence raise circulating angiotensin II concentrations. An elevation in both noradrenaline and angiotensin II plasma levels could be responsible for the reduced responsiveness of α_{1A}-adrenoreceptors which is similar to that previously reported in a fructose fed rat model of LVH [62]. An elevated angiotensin II has been found to be responsible for the suppression of NO-cGMP production [59] as observed in the present study and in cardiac hypertrophy [58]. In order to provide further support for the role of NO in determining the responsiveness of α_{1A}-adrenoreceptors to adrenergic agonists, rats were provided with exogenous L-arginine (NO donor) to enhance the eNOS/NO/cGMP signalling cascade. In the LVH this resulted in heightened vasoconstrictor responses to the α_{1A}agonists NA, PE and ME even when the receptors were blocked when compared to the same phases in the LVH group as shown in Fig 3A, 3B and 3C). These heightened responses were accompanied at the same time by an up regulation of the eNOS/NO/cGMP pathway in the renal cortex. These findings established an association between the up regulated eNOS/NO/cGMP pathway in the renal cortex and heightened responsiveness of α_{1A}-adrenoreceptors to adrenergic agonists in LVH rats treated with L-arginine.

The administration of both the low and high doses of CEC, an α_{1B} $_{v}$adrenoreceptor antagonist, had no effect on the renal vasoconstrictor responses to NA and PE in the Control rats. This was taken to indicate that α_{1B} adrenoreceptors were not functionally contributing to the



* vs. Saline phase of respective group; # vs. Low dose phase of respective group

ϕ vs. respective phase of Control group; Ψ vs. respective phase of LVH

Fig 5. Showing the overall % drop in renal vasoconstrictor responses of α_{1D} adrenoreceptors to NA (5A), PE (5B) and ME (5C) in the kidney of Control, LVH, Control-NO and LVH-NO rats. Data is shown as ± SEM while significance is taken as $p<0.05$.

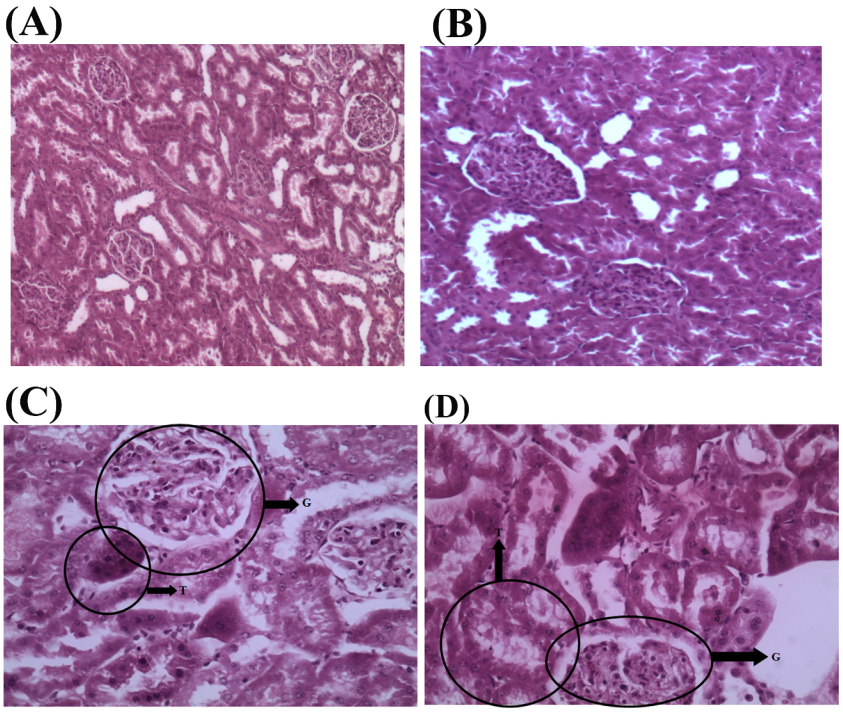
https://doi.org/10.1371/journal.pone.0189386.g005

renal vasoconstriction. These observations support previous studies [45, 63, 64] which found that in normal rats with no renal impairment, there was no functional contribution of α_{1B} adrenoreceptor in mediating the adrenergically induced renal vasoconstriction. However, there were blunted renal vasoconstrictor responses to ME in the Control rats following both the low and high dose CEC phases. This pattern of non-responsiveness to NA and PE but not ME suggested a functional shift of adrenergic receptors with renal vasoconstriction in control rats being mediated by either α_{1A} or α_{1D} adrenoreceptors. The observation that there was reduced renal cGMP levels in the LVH rats suggested an alteration in the G-protein 2nd messenger pathway utilized by α_1 adrenoreceptors which could be responsible, in part, for reduced responsiveness of these receptors. This view was supported by showing that exogenous administration of NO improved renal cGMP levels in the LVH rats consistent with an upregulation of one of the components of this G-protein 2nd messenger pathway which would contribute to the augmented responsiveness of α_{1B} adrenoreceptor to NA, PE and ME in both basal states and following CEC. It is noteworthy that the functional responses of α_{1B} adrenoreceptors to NA, PE and ME in the LVH-NO group were increased in all three phases when compared to same phases of LVH.

It was evident that in the LVH, the responsiveness of α_{1D} adrenoreceptor activation by NA, PE and ME was blunted in the presence of BMY7378. There was a decrease in the magnitude of responses but they were not completely blocked which indicated a functional contribution of α_{1D} adrenoreceptors in LVH. In the present study there were 65%, 50% and 77% increases in the renal vasoconstrictor responsiveness to NA in the saline phase, low and high dose phases of BMY in the LVH-NO compared to the LVH which strengthens the hypothesis of increased responsiveness of α_1 adrenoreceptor subtypes in LVH after treatment with L-arginine. The augmented responses of α_{1D} adrenoreceptor activation to adrenergic agonists in the LVH-NO following blockade with BMY7378 indicated a functional involvement and increased responsiveness of this α_{1D} adrenoreceptor subtype in LVH-NO. As PE is a non-selective agonist for all $\alpha_1$adrenoreceptor subtypes, the fact that in the exogenous administration of PE in LVH-NO resulted in increased responsiveness to α_{1D} adrenoreceptor activation, by 104% and 131% in the low and high dose BMY7387 phases, respectively, compared to those obtained in the LVH indicated an enhanced involvement of the α_{1D} adrenoreceptor subtype. However, administration of the more selective agonist of α_{1D} adrenoreceptor, ME in the LVH-NO group resulted in augmented responses, of 128% and 254% following blockade with BMY7378 which indicated that the functional contribution of this adrenergic receptor subtype was elevated under these conditions.

The exact mechanism by which exogenous administration of L-arginine increased the responsiveness of $\alpha_1$adrenoreceptors in LVH is not clear but it is likely to be of multifactorial origin. There is a view arising from a number of other reports that there is an increased contribution from spare receptors [65, 66].An alternative suggestion, arising from the findings is that exogenous administration of NO donor in LVH up regulated the α_1-adrenoreceptors whereas in pathophysiological states associated with prolonged hyper sympathetic activity, α_1-adrenoreceptors have been reported to be down regulated [67] mostly in the renal vasculature [68]. Inhibitors of NO increased renal sympathetic nerve activity [69], the observations of the present study support the concept of a decreased renal sympathetic activity following exogenous administration of NO which could be responsible for the enhanced responsiveness of α_1-adrenoreceptors to the adrenergic agonists in LVH-NO. A limitation of the present study

Histopathology of kidney tissues using H& E staining



Histopathology of kidney tissues using PicroSirius red staining

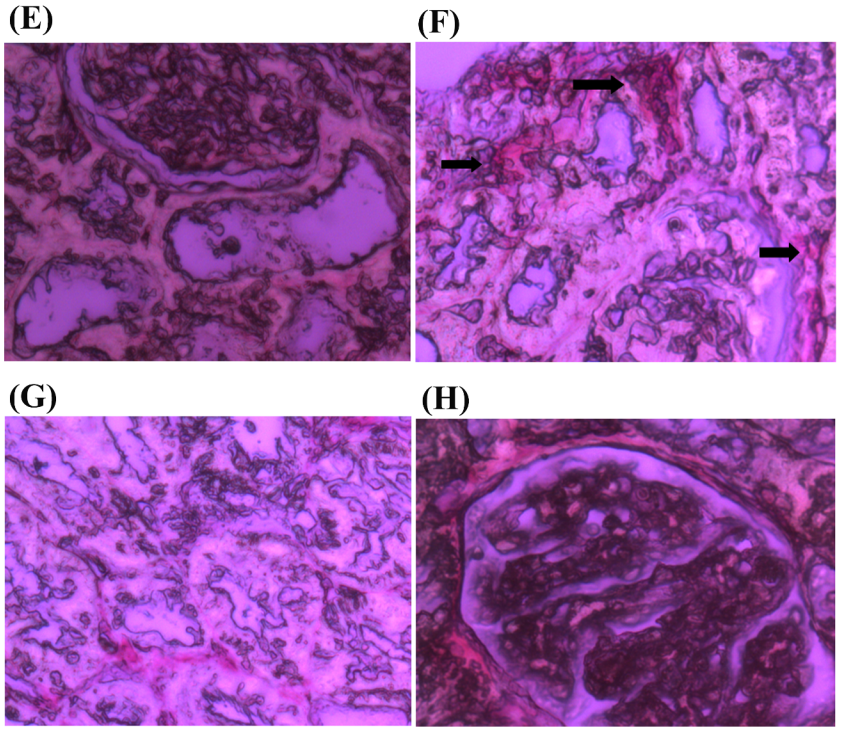


Fig 6. Histopathological study of kidney tissue of Control (A), LVH-WKY (B), Control-NO (C) and LVH-NO (D) for H& E staining while Control (E), LVH-WKY (F), Control-NO (G) and LVH-NO (H) for PicroSirius red staining. Fig 6 shows G and T which represents changes in glomerulus and tubules shape in treatment groups while black arrows pointing out the area of the kidney with collagen plaques.

https://doi.org/10.1371/journal.pone.0189386.g006

was that the expression of α_1-adrenoreceptors in the kidney was not determined. Nonetheless, the sensitivity of these receptors was decreased at a time of elevated renal sympathetic activity in the LVH rats.

More promising evidence for an increased responsiveness was the observations of the modulation of the eNOS/NO/cGMP pathway which is part of G-protein coupled receptor 2nd messenger pathway system. This system was down regulated in LVH but up regulated following elevation of the signalling cascade with L-arginine which demonstrated a clear association between the responsiveness of α_1-adrenoreceptors and the level of expression of the eNOS/NO/cGMP pathway. L-arginine and α_1-adrenoreceptors acts through G-protein pathway so it was assumed that upregulation of cGMP pathway is expected to upregulate or increase the responsiveness of the α_1-adrenoreceptors which are desensitized in LVH and reason for the selection of L-arginine in this study.

It was apparent that there was an interaction between H_2S and NO as there was a negative impact of the NO donor on renal CSE mRNA expression in LVH rats. These findings contrast with previous reports [27, 70] which concluded that NO was essential for H_2S production but they are consistent with the suggestion that in normal circumstances where an NO donor enhances plasma concentrations of H_2S but has an insignificant impact on renal expression of CSE mRNA. This increased H_2S production in plasma may be due to other H_2S producing enzymes like *cystathione beta synthase* (CBS). It is possible to conclude from the present findings that there is an interaction between CSE/H_2S and eNOS/NO under normal conditions but it is abolished in the kidney in LVH. This point of contention is in line with previously reported study [71].

Conclusion

In summary, the present study explored whether there was a down regulation of eNOS/NO/cGMP pathway in the kidney of LVH rats. It was found that exogenous administration of a NO precursor (L-arginine) in LVH not only increased the renal cortical blood perfusion but also enhanced the blunted responsiveness of α_1-adrenoreceptors subtypes to adrenergic agonists by up regulating the eNOS/NO/cGMP pathway in the kidney. We also explored whether there was an interaction between the CSE/H_2S and ENOS/NO cascades under normal conditions but it became apparent that this mutual interaction was abolished in the kidneys of rats with LVH.

Supporting information

S1 Fig. Effects of NA on the responsiveness of α_{1A}–adrenorecptors to adrenoreceptor in Control, LVH, Control-NO and LVH-NO groups. * P<0.05 vs. Saline phase; # P<0.05 vs. Low dose MeU.
(DOC)

S2 Fig. Effects of PE on the responsiveness of α_{1A}–adrenorecptors to adrenoreceptor in Control, LVH, Control-NO and LVH-NO groups. * P<0.05 vs. Saline phase; # P<0.05 vs. Low dose MeU.
(DOC)

S3 Fig. Effects of ME on the responsiveness of α_{1A}–adrenorecptors to adrenoreceptor in Control, LVH, Control-NO and LVH-NO groups. * P<0.05 vs. Saline phase; # P<0.05 vs. Low dose MeU.
(DOC)

S4 Fig. Effects of NA on the responsiveness of α_{1B}–adrenorecptors to adrenoreceptor in Control, LVH, Control-NO and LVH-NO groups. * P<0.05 vs. Saline phase; # P<0.05 vs. Low dose CEC.
(DOC)

S5 Fig. Effects of PE on the responsiveness of α_{1B}–adrenorecptors to adrenoreceptor in Control, LVH, Control-NO and LVH-NO groups. * P<0.05 vs. Saline phase; # P<0.05 vs. Low dose CEC.
(DOC)

S6 Fig. Effects of ME on the responsiveness of α_{1B}–adrenorecptors to adrenoreceptor in Control, LVH, Control-NO and LVH-NO groups. * P<0.05 vs. Saline phase; # P<0.05 vs. Low dose CEC.
(DOC)

S7 Fig. Effects of NA on the responsiveness of α_{1D}–adrenorecptors to adrenoreceptor in Control, LVH, Control-NO and LVH-NO groups. * P<0.05 vs. Saline phase; # P<0.05 vs. Low dose BMY.
(DOC)

S8 Fig. Effects of PE on the responsiveness of α_{1D}–adrenorecptors to adrenoreceptor in Control, LVH, Control-NO and LVH-NO groups. * P<0.05 vs. Saline phase; # P<0.05 vs. Low dose BMY.
(DOC)

S9 Fig. Effects of ME on the responsiveness of α_{1D}–adrenorecptors to adrenoreceptor in Control, LVH, Control-NO and LVH-NO groups. * P<0.05 vs. Saline phase; # P<0.05 vs. Low dose BMY.
(DOC)

S1 Table. Heart index, LV index, R-amplitude and QRS complex of Control WKY, LVH-WKY, Control-WKY and LVH-WKY groups. Heart index, LV index, R-amplitude and QRS complex of Control WKY, LVH-WKY, Control-WKY and LVH-WKY groups on days 35. The values are mean±SEM (n = 6).P<0.05.Statistical analysis was done by one-way analysis of variance followed by Bonferroni *post hoc* test for all the groups. * vs. Control WKY D-35; # vs. LVH-WKY D-35.
(DOC)

Acknowledgments

The Institute of Postgraduate Studies (IPS) is acknowledged for the provision of a USM fellowship (Teaching) to Ashfaq Ahmad (APEX (1002/JHEA/ATSG4001). The authors fully acknowledge USM-RU grant no. 1001/PFARMASI/815078 for this work. All the authors have no conflict of interest.

Author Contributions

Conceptualization: Ashfaq Ahmad.

Formal analysis: Owais Bhatt.

Methodology: Ashfaq Ahmad, Maleeha Azam, Safia A. Khan.

Project administration: Ashfaq Ahmad.

Supervision: Munavvar A. Sattar.

Writing – original draft: Ashfaq Ahmad, Edward J. Johns.

Writing – review & editing: Maleeha Azam, Edward J. Johns.

References

1. Greenwood JP, Scott EM, Stoker JB, Mary DA. Hypertensive left ventricular hypertrophy: relation to peripheral sympathetic drive. J Am Coll Cardiol. 2001; 38(6):1711–7. PMID: 11704385
2. Shokoji T, Nishiyama A, Fujisawa Y, Hitomi H, Kiyomoto H, Takahashi N, et al. Renal sympathetic nerve responses to tempol in spontaneously hypertensive rats. Hypertension. 2003; 41(2):266–73. PMID: 12574093
3. Sun C-L, Hanig J. Vascular reactivity to adrenergic agents and neuronal and vascular catecholamine levels in spontaneously hypertensive rats. Pharmacology. 1983; 27(6):319–24. PMID: 6143329
4. Hye Khan MA, Sattar MA, Abdullah NA, Johns EJ. Influence of combined hypertension and renal failure on functional α1-adrenoceptor subtypes in the rat kidney. Br J Pharmacol. 2008; 153(6):1232–41. https://doi.org/10.1038/bjp.2008.13 PMID: 18246093
5. Ahmad A, Sattar MA, Rathore HA, Abdulla MH, Khan SA, Abdullah NA, et al. Functional contribution of α1D-adrenoceptors in the renal vasculature of left ventricular hypertrophy induced with isoprenaline and caffeine in Wistar-Kyoto rats. Can J Physiol Pharmacol. 2014; 92(12):1029–35. https://doi.org/10.1139/cjpp-2014-0236 PMID: 25403946
6. Bell DG, Jacobs I, Ellerington K. Effect of caffeine and ephedrine ingestion on anaerobic exercise performance. Med Sci Sports Exerc. 2001; 33(8):1399–403. PMID: 11474345
7. Collomp K, Ahmaidi S, Audran M, Chanal J, Prefaut C. Effects of caffeine ingestion on performance and anaerobic metabolism during the Wingate Test. Int J Sports Med. 1991; 12(5):439–43. https://doi.org/10.1055/s-2007-1024710 PMID: 1752708
8. Salomonsson M, Arendshorst WJ. Norepinephrine-induced calcium signaling pathways in afferent arterioles of genetically hypertensive rats. American Journal of Physiology-Renal Physiology. 2001; 281(2): F264–F72. https://doi.org/10.1152/ajprenal.2001.281.2.F264 PMID: 11457717
9. Guimarães S, Moura D. Vascular adrenoceptors: an update. Pharmacol Rev. 2001; 53(2):319–56. PMID: 11356987
10. Fiedler B, Lohmann SM, Smolenski A, Linnemüller S, Pieske B, Schröder F, et al. Inhibition of calcineurin-NFAT hypertrophy signaling by cGMP-dependent protein kinase type I in cardiac myocytes. Proceedings of the National Academy of Sciences. 2002; 99(17):11363–8.
11. Liang M, Knox FG. Production and functional roles of nitric oxide in the proximal tubule. American Journal of Physiology-Regulatory, Integrative and Comparative Physiology. 2000; 278(5):R1117–R24. https://doi.org/10.1152/ajpregu.2000.278.5.R1117 PMID: 10801277
12. Ortiz PA, Garvin JL. Role of nitric oxide in the regulation of nephron transport. American Journal of Physiology-Renal Physiology. 2002; 282(5):F777–F84. https://doi.org/10.1152/ajprenal.00334.2001 PMID: 11934686
13. Tripatara P, Patel NS, Webb A, Rathod K, Lecomte FM, Mazzon E, et al. Nitrite-derived nitric oxide protects the rat kidney against ischemia/reperfusion injury in vivo: role for xanthine oxidoreductase. J Am Soc Nephrol. 2007; 18(2):570–80. https://doi.org/10.1681/ASN.2006050450 PMID: 17202421
14. Chander V, Chopra K. Protective effect of nitric oxide pathway in resveratrol renal ischemia-reperfusion injury in rats. Arch Med Res. 2006; 37(1):19–26. https://doi.org/10.1016/j.arcmed.2005.05.018 PMID: 16314181
15. Mattson DL, Roman RJ, Cowley A. Role of nitric oxide in renal papillary blood flow and sodium excretion. Hypertension. 1992; 19(6 Pt 2):766–9. PMID: 1592478
16. Zou A-P, Cowley AW. Nitric Oxide in Renal Cortex and Medulla An In Vivo Microdialysis Study. Hypertension. 1997; 29(1):194–8.
17. Furchgott RF, Zawadzki JV. The obligatory role of endothelial cells in the relaxation of arterial smooth muscle by acetylcholine. Nature. 1980; 288(5789):373–6. PMID: 6253831

18. Goligorsky MS, Brodsky SV, Noiri E. Nitric oxide in acute renal failure: NOS versus NOS. Kidney Int. 2002; 61(3):855–61. https://doi.org/10.1046/j.1523-1755.2002.00233.x PMID: 11849438

19. AHMAD Ashfaq SMA, RATHORE Hassaan A, Khan Safia A, Abdullah Norazizan, John Edward J. Down regulation of cystathione γ lyase (CSE) and endothelial nitric oxide synthase (eNOS) and reduced responsiveness of α1A adrenergic receptors in the kidney of left ventricular hypertrophied Wistar Kyoto rats (10.3906/biy-1506-78). Turkish Journal of Biology. 2016; 40(6):1129–39. https://doi.org/10.3906/biy-1506-78

20. Ali M, Ping C, Mok YY, Ling L, Whiteman M, Bhatia M, et al. Regulation of vascular nitric oxide in vitro and in vivo; a new role for endogenous hydrogen sulphide? Br J Pharmacol. 2006; 149(6):625–34. https://doi.org/10.1038/sj.bjp.0706906 PMID: 17016507

21. Hosoki R, Matsuki N, Kimura H. The possible role of hydrogen sulfide as an endogenous smooth muscle relaxant in synergy with nitric oxide. Biochem Biophys Res Commun. 1997; 237(3):527–31. https://doi.org/10.1006/bbrc.1997.6878 PMID: 9299397

22. Grossi L. Hydrogen sulfide: a neurotransmitter or just a cofactor of the nitrite in the NO production? 2008.

23. Yong Q-C, Cheong JL, Hua F, Deng L-W, Khoo YM, Lee H-S, et al. Regulation of heart function by endogenous gaseous mediators—crosstalk between nitric oxide and hydrogen sulfide. Antioxidants & redox signaling. 2011; 14(11):2081–91.

24. Whiteman M, Moore PK. Hydrogen sulfide and the vasculature: a novel vasculoprotective entity and regulator of nitric oxide bioavailability? J Cell Mol Med. 2009; 13(3):488–507. https://doi.org/10.1111/j.1582-4934.2009.00645.x PMID: 19374684

25. Hosoki R MNaKH. The possible role of hydrogen sulfide as an endogenous smooth muscle relaxant in synergy with nitric oxide. Biochem Biophys Res Commun. 1997; 237:527–31. https://doi.org/10.1006/bbrc.1997.6878 PMID: 9299397

26. Grossi L. Hydrogen sulfide induces nitric oxide release from nitrite. Bioorg Med Chem Lett. 2009; 19 (21):6092–4. https://doi.org/10.1016/j.bmcl.2009.09.030 PMID: 19782565

27. Zhao W, Ndisang JF, Wang R. Modulation of endogenous production of H_2S in rat tissues. Can J Physiol Pharmacol. 2003; 81(9):848–53. https://doi.org/10.1139/y03-077 PMID: 14614520

28. Wang R. Two's company, three'sa crowd: can H2S be the third endogenous gaseous transmitter? The FASEB Journal. 2002; 16(13):1792–8. https://doi.org/10.1096/fj.02-0211hyp PMID: 12409322

29. Shen J, Ma S, Chan P, Lee W, Fung PC, Cheung RT, et al. Nitric oxide down-regulates caveolin-1 expression in rat brains during focal cerebral ischemia and reperfusion injury. J Neurochem. 2006; 96 (4):1078–89. https://doi.org/10.1111/j.1471-4159.2005.03589.x PMID: 16417587

30. Flanagan ET, Buckley MM, Aherne CM, Lainis F, Sattar M, Johns EJ. Impact of cardiac hypertrophy on arterial and cardiopulmonary baroreflex control of renal sympathetic nerve activity in anaesthetized rats. Exp Physiol. 2008; 93(9):1058–64. https://doi.org/10.1113/expphysiol.2008.043216 PMID: 18487313

31. Ye S, Nosrati S, Campese VM. Nitric oxide (NO) modulates the neurogenic control of blood pressure in rats with chronic renal failure (CRF). J Clin Invest. 1997; 99(3):540. https://doi.org/10.1172/JCI119191 PMID: 9022090

32. Hassan MI, Boosen M, Schaefer L, Kozlowska J, Eisel F, von Knethen A, et al. Platelet-derived growth factor-BB induces cystathionine γ-lyase expression in rat mesangial cells via a redox-dependent mechanism. Br J Pharmacol. 2012; 166(8):2231–42. https://doi.org/10.1111/j.1476-5381.2012.01949.x PMID: 22428706

33. Xu S, Zhou X, Yuan D, Xu Y, He P. Caveolin-1 scaffolding domain promotes leukocyte adhesion by reduced basal endothelial nitric oxide-mediated ICAM-1 phosphorylation in rat mesenteric venules. American Journal of Physiology-Heart and Circulatory Physiology. 2013; 305(10):H1484–H93. https://doi.org/10.1152/ajpheart.00382.2013 PMID: 24043249

34. Lee D-Y, Wauquier F, Eid AA, Roman LJ, Ghosh-Choudhury G, Khazim K, et al. Nox4 NADPH Oxidase Mediates Peroxynitrite-dependent Uncoupling of Endothelial Nitric-oxide Synthase and Fibronectin Expression in Response to Angiotensin II ROLE OF MITOCHONDRIAL REACTIVE OXYGEN SPECIES. J Biol Chem. 2013; 288(40):28668–86. https://doi.org/10.1074/jbc.M113.470971 PMID: 23940049

35. Santha P, Pakaski M, Fazekas O, Szucs S, Fodor E, Kálmán J Jr, et al. [Acute and chronic stress induced changes in gene transcriptions related to Alzheimer's disease]. Ideggyogyaszati szemle. 2012; 65(5–6):195–200. PMID: 22724288

36. Cannino G, Ferruggia E, Rinaldi AM. Proteins participating to the post-transcriptional regulation of the mitochondrial cytochrome c oxidase subunit IV via elements located in the 3′ UTR. Mitochondrion. 2009; 9(6):471–80. https://doi.org/10.1016/j.mito.2009.08.007 PMID: 19703590

37. Livak KJ, Schmittgen TD. Analysis of Relative Gene Expression Data Using Real-Time Quantitative PCR and the 2−ΔΔCT Method. Methods. 2001; 25(4):402–8. http://dx.doi.org/10.1006/meth.2001.1262. https://doi.org/10.1006/meth.2001.1262 PMID: 11846609

38. Ohashi Y, Kawashima S, i Hirata K, Yamashita T, Ishida T, Inoue N, et al. Hypotension and reduced nitric oxide-elicited vasorelaxation in transgenic mice overexpressing endothelial nitric oxide synthase. J Clin Invest. 1998; 102(12):2061. https://doi.org/10.1172/JCI4394 PMID: 9854041

39. Ahmad A, Sattar M, Rathore H, Abdulla M, Khan S, Abdullah N, et al. Enhanced expression of endothelial nitric oxide synthase in the myocardium ameliorates the progression of left ventricular hypertrophy in l-arginine treated wistar-kyoto rats. JPP. 2016;(2):03.

40. Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem. 1976; 72(1):248–54.

41. Yan H, Du J, Tang C. The possible role of hydrogen sulfide on the pathogenesis of spontaneous hypertension in rats. Biochem Biophys Res Commun. 2004; 313(1):22–7. PMID: 14672692

42. Ahmad A, Sattar MA, Azam M, Abdulla MH, Khan SA, Hashmi F, et al. Cystathione gamma lyase/Hydrogen Sulphide Pathway Up Regulation Enhances the Responsiveness of α1A and α1B-Adrenoreceptors in the Kidney of Rats with Left Ventricular Hypertrophy. PLoS ONE. 2016; 11(5):e0154995. https://doi.org/10.1371/journal.pone.0154995 PMID: 27191852

43. Sattar MA, Johns EJ. Evidence for an alpha Adrenoceptor Subtype Mediating Adrenergic Vasoconstriction in Wistar Normotensive and Stroke-Prone Spontaneously Hypertensive Rat Kidney. J Cardiovasc Pharmacol. 1994; 23(2):232–9. PMID: 7511752

44. Abdulla MH, Sattar MA, Johns EJ, Abdullah NA, Abdul Hye Khan M, Rathore HA. High-fructose feeding impacts on the adrenergic control of renal haemodynamics in the rat. Br J Nutr. 2012; 107(2):218. https://doi.org/10.1017/S0007114511002716 PMID: 21733307

45. Armenia A, Munavvar A.S., Abdullah N.A., Helmi A. & Johns E.J. The contribution of adrenoceptor subtype (s) in the renal vasculature of diabetic spontaneously hypertensive rats Br J Pharmacol 2004; 142:719–26. Epub 726. https://doi.org/10.1038/sj.bjp.0705842 PMID: 15172958

46. Dibona GF, Kopp UC. Neural control of renal function. Physiol Rev. 1997; 77(1):75–197. https://doi.org/10.1152/physrev.1997.77.1.75 PMID: 9016301

47. Abdulla M, Sattar M, Abdullah N, Khan MA, Anand Swarup KL, Johns E. The contribution of α1B-adrenoceptor subtype in the renal vasculature of fructose-fed Sprague–Dawley rats. Eur J Nutr. 2011; 50 (4):251–60. https://doi.org/10.1007/s00394-010-0133-8 PMID: 20882287

48. Khan H, Sattar M, Abdullah N, Johns E. Influence of combined hypertension and renal failure on functional α1-adrenoceptor subtypes in the rat kidney. Br J Pharmacol. 2008; 153(6):1232–41. https://doi.org/10.1038/bjp.2008.13 PMID: 18246093

49. Abdulla M, Sattar M, Johns E, Abdullah N, Khan MA. Evidence for the role of α1A-adrenoceptor subtype in the control of renal haemodynamics in fructose-fed Sprague–Dawley rat. Eur J Nutr. 2011; 50 (8):689–97. https://doi.org/10.1007/s00394-011-0180-9 PMID: 21373947

50. Ahmad A, Sattar MA, Rathore HA, Abdulla MH, Khan SA, Abdullah NA, et al. Functional contribution of α1D-adrenoceptors in the renal vasculature of left ventricular hypertrophy induced with isoprenaline and caffeine in Wistar–Kyoto rats. Can J Physiol Pharmacol. 2014; 92(12):1029–35. https://doi.org/10.1139/cjpp-2014-0236 PMID: 25403946

51. Gross G, Hanft G, Rugevics C. 5-Methyl-urapidil discriminates between subtypes of the α 1-adrenoceptor. Eur J Pharmacol. 1988; 151(2):333–5. PMID: 2901974

52. Nunes J, Guimaraes S. Chloroethylclonidine irreversibly activates postjunctional alpha2-adrenoceptors in the dog saphenous vein. Naunyn Schmiedebergs Arch Pharmacol. 1993; 348(3):264–8. PMID: 7901777

53. Goetz AS, King HK, Ward SD, True TA, Rimele TJ, Saussy DL. BMY 7378 is a selective antagonist of the D subtype of α 1-adrenoceptors. Eur J Pharmacol. 1995; 272(2):R5–R6.

54. Bode-Boger S, Boger R, Creutzig A, Tsikas D, Gutzki F, Alexander K, et al. L-arginine infusion decreases peripheral arterial resistance and inhibits platelet aggregation in healthy subjects. Clin Sci. 1994; 87(3):303–10. PMID: 7955906

55. Busse R, Fleming I. Endothelial dysfunction in atherosclerosis. J Vasc Res. 1996; 33(3):181–94. PMID: 8924517

56. Palmer RM, Ferrige A, Moncada S. Nitric oxide release accounts for the biological activity of endothelium-derived relaxing factor. 1987. https://doi.org/10.1038/327524a0 PMID: 3495737

57. Shah AM, MacCarthy PA. Paracrine and autocrine effects of nitric oxide on myocardial function. Pharmacol Ther. 2000; 86(1):49–86. PMID: 10760546

58. Booz GW. Putting the Brakes on Cardiac Hypertrophy Exploiting the NO–cGMP Counter-Regulatory System. Hypertension. 2005; 45(3):341–6. https://doi.org/10.1161/01.HYP.0000156878.17006.02 PMID: 15710777

59. Mollnau H, Wendt M, Szöcs K, Lassègue B, Schulz E, Oelze M, et al. Effects of angiotensin II infusion on the expression and function of NAD (P) H oxidase and components of nitric oxide/cGMP signaling. Circ Res. 2002; 90(4):e58–e65. PMID: 11884382

60. Elhawary AM, Pettinger WA, Wolff DW. Subtype-selective alpha-1 adrenoceptor alkylation in the rat kidney and its effect on the vascular pressor response. J Pharmacol Exp Ther. 1992; 260(2):709–13. PMID: 1346641

61. Hogikyan RV, Supiano MA. Arterial alpha-adrenergic responsiveness is decreased and SNS activity is increased in older humans. American Journal of Physiology-Endocrinology And Metabolism. 1994; 266 (5):E717–E24.

62. Kobayashi R, Nagano M, Nakamura F, Higaki J, Fujioka Y, Ikegami H, et al. Role of angiotensin II in high fructose-induced left ventricular hypertrophy in rats. Hypertension. 1993; 21(6 Pt 2):1051–5. PMID: 8505091

63. Yatsu T, Aoki M, Inagaki O. Preventive effect of zelandopam, a dopamine D1 receptor agonist, on cisplatin-induced acute renal failure in rats. Eur J Pharmacol. 2003; 461(2):191–5.

64. SATTAR MAJ E.J. (1994b). a1-adrenoceptor subtype mediating adrenergic vasoconstriction in kidney of 2K1C Goldblatt and DOCA-salt hypertensive rats. J Cardiovasc Pharmacol. 24:420–8.

65. Piascik MT, Sparks MS, Pruitt TA, Soltis EE. Evidence for a complex interaction between the subtypes of the α 1-adrenoceptor. Eur J Pharmacol. 1991; 199(3):279–89. PMID: 1680715

66. Arévalo-León LE, Gallardo O, amp, x, z IA, Urquiza M, et al. Evidence for the role of α1D- and α1A-adrenoceptors in contraction of the rat mesenteric artery. Vascul Pharmacol. 2003; 40(2):91–6. http://dx.doi.org/10.1016/S1537-1891(02)00336-1. PMID: 12646397

67. Adolfo García-Sáinz J. α1-adrenergic action: Receptor subtypes, signal transduction and regulation. Cell Signal. 1993; 5(5):539–47. http://dx.doi.org/10.1016/0898-6568(93)90049-R. PMID: 8312131

68. Khan MAH, Sattar MA, Abdullah NA, Johns EJ. α1B-Adrenoceptors mediate adrenergically-induced renal vasoconstrictions in rats with renal impairment. Acta Pharmacol Sin. 2008; 29(2):193–203. https://doi.org/10.1111/j.1745-7254.2008.00727.x PMID: 18215348

69. Sakuma I, Togashi H, Yoshioka M, Saito H, Yanagida M, Tamura M, et al. NG-methyl-L-arginine, an inhibitor of L-arginine-derived nitric oxide synthesis, stimulates renal sympathetic nerve activity in vivo. A role for nitric oxide in the central regulation of sympathetic tone? Circ Res. 1992; 70(3):607–11. https://doi.org/10.1161/01.res.70.3.607 PMID: 1537096

70. Yanfei W, Lin S, Junbao D, Chaoshu T. Impact of l-arginine on hydrogen sulfide/cystathionine-γ-lyase pathway in rats with high blood flow-induced pulmonary hypertension. Biochem Biophys Res Commun. 2006; 345(2):851–7. http://dx.doi.org/10.1016/j.bbrc.2006.04.162. https://doi.org/10.1016/j.bbrc.2006.04.162 PMID: 16701554

71. Testai L, D'Antongiovanni V, Piano I, Martelli A, Citi V, Duranti E, et al. Different patterns of H2S/NO activity and cross-talk in the control of the coronary vascular bed under normotensive or hypertensive conditions. Nitric Oxide. 2015; 47:25–33. http://dx.doi.org/10.1016/j.niox.2015.03.003. https://doi.org/10.1016/j.niox.2015.03.003 PMID: 25795591