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Enhanced A₁ adenosine receptor-induced vascular contractions in mesenteric artery and aorta of in L-NAME mouse model of hypertension.

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Abstract

L-NAME-induced hypertension is commonly used to study endothelial dysfunction and related vascular effects. It has been reported that genetic deletion of A₁ adenosine receptor (AR) reduces blood pressure (BP) increases in mice and thus, suggesting the involvement of A₁AR. Thus, we sought to determine whether A₁AR-induced vascular responses were altered in this mouse model of hypertension. L-NAME (1 mg/ml) was given in the drinking water for 28 days to mice. The BP was monitored using non-invasive tail-cuff system. Muscle tension studies were performed using DMT for mesenteric arteries (MAs) and organ bath for aorta. Protein expression was analyzed by western blot. Significantly, higher systolic and mean arterial blood pressure was noted in L-NAME mice. In MAs, higher 2-Chloro-N⁶-cyclopentyladenosine (CCPA, selective A₁AR agonist) induced contractions in hypertensive mice were observed. This enhanced contraction was inhibited by HET0016 (Cytochrome 450 4A inhibitor, 10 μM, 15 min). Contrary, 5'-(N-Ethylcarboxamido) adenosine (NECA, non-selective AR agonist) induced vascular responses were comparable in both groups. Pinacidil (K_{ATP} channel opener) induced relaxation was significantly increased in hypertensive mice. In aorta, CCPA-induced contractions were enhanced and inhibited by HET0016 in hypertensive mice. Notably, NECA-induced contractions in aorta were enhanced in hypertensive mice. Higher expressions of A₁AR and Cyp4A were noted in MAs of hypertensive mice. In addition, in aorta, higher A₁AR and comparable Cyp4A levels were observed in hypertensive mice. A₁AR-induced vascular contractions were enhanced in hypertensive mice aorta and MAs. Cyp4A plays a role in altered vascular responses in MAs.

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Authors contributions:

VY and SJM conceived and designed research; VY and BT performed experiments and analyzed data; VY drafted the manuscript; VY, BT and SJM revised and edited the manuscript.

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Disclosures

None.

Conflicts of interest:

The authors declare no conflicts of interest.

Keywords

L-NAME hypertension; A₁AR; aorta; MAs; Cyp4A

1. Introduction

Hypertension, characterized by high blood pressure (BP) is involved in various cardiovascular-related diseases. Endothelial dysfunction related to reducing NO levels in the circulation is an important contributing factor responsible for hypertension (Konishi and Su, 1983; Laughlin et al., 2012; Luscher et al., 1988; Nguyen Dinh Cat and Touyz, 2011; Tian et al., 2011). L-NAME, a non-specific NOS inhibitor, down regulates endothelial nitric oxide synthase (eNOS) protein expression and leads to depletion of plasma nitric oxide (NO). L-NAME pre-treatment causes systemic vasoconstriction, increased vascular resistance and high BP (Berkban et al., 2015; Nishijima et al., 2014; Paulis et al., 2008). Since, we were interested to study the role of NO and BP regulation and its relationship to vascular ARs, we chose L-NAME model of hypertension for this study.

Adenosine is an important metabolite which regulates local blood flow by controlling vascular tone through activating four subtypes of adenosine receptors (ARs). These ARs are G-protein coupled receptors (GPCRs) namely A₁, A_{2A}, A_{2B}, and A₃. A₁ and A₃ AR activation cause vascular contraction, while A_{2A} and A_{2B} AR stimulation produces vascular relaxation (Hein et al., 2013; Jacobson and Gao, 2006; Mubagwa and Flameng, 2001; Mubagwa et al., 1996; Ohisalo, 1987; Olanrewaju and Mustafa, 2000; Olanrewaju et al., 2000). In mouse aorta, A₁AR-dependent smooth muscle contraction involves cytochrome P450 epoxygenase type 4A (Cyp4A), protein kinase C- α , and mitogen-activated protein kinases, mainly p44/42 MAPK (Ansari et al., 2009; Kunduri et al., 2013a; Kunduri et al., 2013b; Sanjani et al., 2011). Notably, A₁AR-induced vascular contractions were endothelium-dependent in mouse aorta (Nayeem et al., 2008; Ponnoth et al., 2012b). A₃AR-dependent contraction occurs through the generation of thromboxane B₂ by cyclooxygenases-1 that requires the presence of intact endothelium in mouse aorta (Ansari et al., 2007). In mouse MAs, A₁AR mediates contraction while the A_{2B}AR mediates relaxation (Teng et al., 2013). A₁AR dependent vascular contractions involve activation of Cyp4A, which was dependent on the presence of intact endothelium. Moreover, non-selective AR (such as, NECA)-induced vascular relaxations were partly dependent on endothelium and K_{ATP} channel activity (Yadav et al., 2015). Thus, endothelium plays an important role in ARs induced vascular responses.

Reportedly, angiotensin II stimulation-enhanced A₁AR-induced vascular contractions in mouse MAs (Yadav et al., 2015). Interestingly, in L-NAME-induced hypertensive mice, genetic deletion of A₁AR (A₁AR^{-/-}) attenuates renal afferent arteriolar contraction and hypertension (Gao et al., 2011). Genetic deletion of A₁AR protected L-NAME and ANG II-induced oxidative stress in arterioles²⁵. Furthermore, arteriolar contractions to ANG II were lower in A₁AR KO mice of L-NAME induced hypertensive mice (Gao et al., 2011). Notably, eNOS expression was lower in A₁AR^{-/-} mice renal afferent arterioles. Suggesting A₁AR may regulate eNOS expression (Gao et al., 2011). In A₁AR^{-/-} mice, we reported that NO

was not involved in enhanced coronary reactive hyperemia (Zhou et al., 2015a). Suggesting A₁AR may negatively regulate coronary reactive hyperemia. Thus, A₁AR may regulate vascular tone in L-NAME induced hypertension.

We sought to determine whether AR-dependent vascular responses (including its signaling through K_{ATP} channels) were altered in L-NAME induced hypertension in mice.

2. Materials and Methods

2.1 Animals

Male (C57 BL/6 background) mice of 14–17 weeks age were used in the present study. All animal care and experimentation protocols were approved and carried out in accordance with the West Virginia University Institutional Animal Care and Use Committee and were in accordance with the principles and guidelines of the NIH's Guide for the Care and Use of Laboratory Animals. Our animal facility is AAALAC approved.

2.2 Pharmacological agents and antibodies

5'-(N-ethylcarboxamido) adenosine (NECA) and 2-chloro-N cyclopentyladenosine (CCPA) were purchased from Tocris Biosciences (Minneapolis, MN). Phenylephrine (PE), acetyl choline (ACh), HET0016 and all other chemicals were purchased from Sigma-Aldrich. Antibodies for CYP4A (sc-271983), eNOS (sc-654) and actin (sc-47778) were purchased from Santa Cruz Biotechnology, Santa Cruz, CA. The A₁AR antibody was purchased from Sigma-Aldrich.

2.3 L-NAME model and Blood pressure measurements

Mice were given L-NAME in drinking water at 1 mg/ml concentration for 28 days. The blood pressure was measured throughout the 28 days protocol using non-invasive tail cuff (CODA-8) method obtained from Kent Scientific (Daugherty et al., 2009) as per manufacturers protocol. Data are presented as both mean arterial blood pressure (MABP) and systolic blood pressure (SABP). Average weight of the mice at the start of the protocol was (in gm) 28.5 ± 0.8 (n=9) and 28.1 ± 0.8 (n=10) in control and L-NAME groups, respectively. At the end of the protocol average weight of the mice in control group was significantly increased to 30.9 ± 1.1 . On the other hand, weight of the mice in L-NAME group was comparable between start and end of the protocol. Drinking water to control and L-NAME groups was provided ad libitum. On an average control mice consumed ~16 ml/week whereas, L-NAME mice consumed ~ 6 ml/week water.

2.4 Tissue preparation and isometric force measurement

Mice were euthanized by anesthesia with pentobarbital sodium (65 mg/kg i.p.). By thoracotomy aorta was dissected out and the mouse mesentery with intestines were carefully excised and placed in cold oxygenated and (5% CO₂ and 95% O₂) modified Krebs–Henseleit physiological salt solution (PSS, in mM: NaCl 120, NaHCO₃ 25, KCl 4.7, KH₂PO₄ 1.2, CaCl₂ 1.8, MgSO₄ 1.2, glucose 15, and EDTA 0.05, pH 7.4).

The aorta was subjected to isometric force measurement using isolated organ bath method as described from our laboratory (Ponnoth et al., 2012a; Zhou et al., 2015b). A preload of 1 gm was given to isolated aorta rings during the equilibration period. A DMT (Danish Myo Technology A/S, Aarhus, Denmark) myograph was used to perform muscle tension measurements in MAs as described previously (Yadav et al., 2016; Yadav et al., 2015). Second order MA rings (3–5 mm long, 75–150 μ m in diameter) were mounted on the DMT myograph with continuous oxygenation at 37°C. A preload of 300 mg was applied to the MAs during the equilibration period of 90 min. In both vascular tissues, the viability of the arteries and the presence of endothelium was tested using potassium chloride (KCl, 50 mM) and acetylcholine (ACh 1 μ M), respectively. Concentration-response curves (CRCs) were obtained in tissues initially contracted with phenylephrine (PE 1 μ M). The muscle tension generated in each CRCs was plotted and normalized to the corresponding % contraction to the basal tension obtained using PE (1 μ M). Intact endothelium was present in all of our muscle tension measurement experiments.

2.5 Western blotting

Western blot analysis was performed as described previously (Ponnoth et al., 2012a; Ponnoth et al., 2012b; Yadav et al., 2016; Yadav et al., 2015). Briefly, aorta and MAs isolated from control and L-NAME mice were homogenized with 125 μ l of RIPA Lysis and Extraction Buffer (Peirce #89901 mixed with 1 mM of Na₃VO₄, 1 mg/ml of leupeptin and 1mM NaF), vortexed, and centrifuged for 10 min at 10,000 g at 4°C. Protein was estimated by Lowry's method using Bio-Rad Laboratories protein assay concentrate. Samples (5–15 μ g of total protein) were loaded into slab gels (10% acrylamide; 1.5 mm thick), separated by sodium dodecyl sulfate polyacrylamide gel electrophoresis, and transferred to nitrocellulose membranes (Hybond-ECL). Membranes were blocked with 5% nonfat dry milk for 45 min and incubated overnight with primary antibodies. All primary antibody dilutions were 1:1000. Membranes were developed using enhanced chemiluminescence (Amersham BioSciences) and GE Amersham Imager 600. Densitometry analysis was performed using Multi Guage V3.0 software. The data obtained were normalized to the intensity of the actin band and presented as the normalized ratio (% change) of the target protein to control protein expression.

2.6 Statistical analysis

All the data are presented as a mean \pm standard error. Data in CRCs were analyzed between groups at the same concentrations. Comparisons between two groups were made using Student's t-test. Two way ANOVA, followed by Bonferroni post-tests, was used to compare means of different CRCs. Values at $P < 0.05$ were considered statistically significant. In all data, "n" represents the number of animals used to perform experiments.

3 Results

3.1 Blood pressure response in L-NAME-induced hypertension model

L-NAME (1 mg/ml) was given to the mice in drinking water for 28 days period. The blood pressure responses were regularly monitored throughout the 28 days model period. As shown in Fig. 1, systolic and mean arterial blood pressure (SABP and MABP) was higher in

L-NAME mice. The MABP of L-NAME mice were higher from day 3 onwards and stayed significantly higher throughout the 28 day period. The MABP observed at day 0 was (mmHg) was 83.9 ± 2.3 in control and 86.4 ± 2.7 in L-NAME group ($P > 0.05$). At the end of our hypertension model (day 28), MABP was 86.6 ± 2.5 and 112.2 ± 2.8 in control and L-NAME group, respectively ($P < 0.05$). Whereas, SABP (Fig. 1A) in control group at day 28 was 110.3 ± 1.9 and in L-NAME group was 134.9 ± 3.1 ($P < 0.05$).

3.2 Vascular responses in L-NAME hypertensive mice

In L-NAME hypertensive and control mice, we measured vascular responses in MAs and aorta. In MAs of L-NAME hypertensive mice, ACh ($1 \mu\text{M}$)-induced relaxation was significantly reduced (% to PE) to -93.5 ± 1.4 ($n=9$) compared to -98.5 ± 0.2 ($n=9$) in control. Importantly, PE ($1 \mu\text{M}$)-induced responses were significantly reduced in L-NAME group from (mg) 278.8 ± 32.8 ($n=9$) compared to 492.1 ± 33.8 ($n=9$) in control ($p < 0.05$). In addition, KCl-induced vascular contractions were comparable in MAs. The contractions observed were 369.7 ± 18.1 ($n=9$) in control and 336.1 ± 21.5 ($n=9$) in L-NAME group.

In aorta of L-NAME hypertensive mice, ACh induced relaxation and KCl-induced contractions were comparable to control. KCl induced contractions observed were 312.6 ± 23.7 and 297.0 ± 19.0 ($n=9$ each) in control and L-NAME mouse aorta. ACh induced relaxation observed in aorta were (% to PE) -55.6 ± 2.7 and -59.1 ± 4.6 ($n=9$). Importantly, PE ($1 \mu\text{M}$)-induced vascular contractions were significantly reduced in aorta of L-NAME hypertensive mice. The contractions observed were (mg) 169.5 ± 1.6 ($n=9$) and 113.5 ± 2.0 ($n=9$) in control and L-NAME hypertensive mice ($P < 0.05$), respectively. Thus, vascular responses in both aorta and MAs were altered in L-NAME hypertensive mice.

3.3 Role of A₁AR in regulating vascular tone in MAs and aorta of L-NAME-induced hypertensive mice

To study the role of ARs in regulating vascular tone in L-NAME induced hypertension model, we performed CCPA (selective AR A₁ agonist) and NECA (non-selective AR agonist) induced CRCs in MAs and aorta of control and L-NAME mice. As shown in the Fig. 2A, CCPA induced contractions were significantly higher in MAs of L-NAME mice. Particularly, CCPA at 100 nM caused contraction (% to PE contraction) of 24.9 ± 14.1 and 89.2 ± 27.4 in control and L-NAME mice MAs, respectively ($P < 0.05$). HET0016 (Cyp4A inhibitor, $10 \mu\text{M}$), pretreatment significantly attenuated hypercontractility observed in MAs of L-NAME mice (Fig. 2A) but had no effect in control tissues. Further, we found that NECA-induced contractions were comparable in control and L-NAME mice MAs (Fig. 2B). Pinacidil (K_{ATP} channel opener)-induced vascular relaxation responses were significantly higher in L-NAME mice MAs (Fig. 2C). At $1 \mu\text{M}$, relaxation observed to pinacidil in MAs was -51.7 ± 9.8 and -76.3 ± 5.9 in control and L-NAME, respectively ($P < 0.05$).

In aorta of L-NAME hypertensive mice, significantly enhanced contraction to CCPA was observed (Fig. 3A). At $1 \mu\text{M}$ CCPA, contractions were 78.9 ± 5.9 and 101.2 ± 8.9 in control and L-NAME mice, respectively (Fig. 3A, $P < 0.05$). Notably, HET0016 preincubation significantly reduced CCPA induced contractions in control as well as L-NAME mouse aorta (Fig. 3A). Furthermore, NECA-induced contractions were altered in aorta of L-NAME mice

(Fig. 3B). Pinacidil-induced relaxation responses were comparable in control and L-NAME mice (Fig. 3C). Thus, suggesting that A₁AR plays a role in enhanced vascular contraction, which was mediated by Cyp4A in MAs and aorta of L-NAME mice.

3.4 Expression profile of A₁AR, eNOS and Cyp4A

To test whether changes in the expression of A₁AR and Cyp4A accounts for the change in vascular responses in L-NAME hypertensive mice, we performed western blot analysis. Our data revealed significantly higher expression of A₁AR and Cyp4A (Fig. 4 A–C) in MAs of L-NAME mice. Furthermore, in aorta of L-NAME mice, higher A₁AR and unchanged Cyp4A expression levels were observed compared to control (Fig. 5 A–C). Notably, eNOS expression was comparable in MAs and aorta of control and L-NAME mice. The eNOS expression was 100.0 ± 13.5 (n=6) and 81.1 ± 19.2 (n=7) in MAs of control and L-NAME mice (Fig. 4D–E), respectively. In aorta of control mice, eNOS expression observed was 100.0 ± 13.9 (n=6) and in L-NAME mice was 70.5 ± 11.6 (n=6, Fig. 5D–E).

Thus, indicating that higher expression of A₁AR and Cyp4A play roles in higher contractile responses observed in L-NAME-induced hypertensive mice.

4. Discussion

The present study was aimed to determine whether ARs induced vascular responses were altered in L-NAME model of hypertension in mice. Important findings of this study were as follows.: 1) A₁AR induced contractile responses were higher in aorta and MAs; 2) A₁AR induced contractile responses were dependent on Cyp4A; 3) Higher expression of A₁AR and Cyp4A was observed in MAs; 4) Higher A₁AR and comparable Cyp4A expression was noted in aorta; 5) Pinacidil induced relaxation was higher in MAs; and finally, 6) NECA-induced vascular contractions were higher in aorta. Overall, our data revealed an important role of A₁AR in regulating vascular tone in L-NAME model of hypertension in mice.

The endothelium is an important regulator of vascular tone by releasing NO, prostacyclin, and endothelium-derived hyperpolarizing factors (Edwards et al., 2010). Endothelial dysfunction characterized by reduced bioavailability of vascular dilating and increased contracting agents is present in hypertension (Konishi and Su, 1983; Laughlin et al., 2012; Luscher et al., 1988; Nguyen Dinh Cat and Touyz, 2011; Tian et al., 2011). L-NAME, an eNOS inhibitor, reduces NO bioavailability thereby increases systemic vascular resistance leading to high BP (Berkban et al., 2015; Nishijima et al., 2014; Paulis et al., 2008). As expected, we found that chronic administration of L-NAME increased SABP and MABP in mice (Fig. 1), making mice hypertensive. Further, our vascular response analysis data revealed that in MAs, PE-induced contraction and ACh induced relaxation was reduced in L-NAME hypertensive mice. In aorta, ACh induced relaxation was comparable and PE-induced contractions were significantly reduced in L-NAME hypertensive mice. KCl-induced contractions were comparable in MAs and aorta. Recently, in L-NAME hypertensive rat aorta, significantly reduced ACh-induced relaxation and higher PE-induced contractions were noted (Prawez et al., 2016). Also, in aorta of L-NAME mice, ACh induced relaxation was reduced (Nagano et al., 2013). Importantly, a decrease in eNOS, phosphorylated eNOS, and NO concentrations were reported in L-NAME hypertensive rat

ventricles and aorta (Sheng et al., 2017; Wang et al., 2017). In a study of N^ω-Nitro-L-arginine (L-NNA) induced hypertensive rats, PE-induced contractions were comparable in aorta and MAs (Kanagy, 1997). Thus, there are discrepancies in the PE and ACh induced vascular responses in L-NAME (or L-NNA) induced hypertensive rats and mice. Our data showed that ACh induced relaxation was partially but significantly reduced in L-NAME MAs and was comparable in aorta. In our MA preparations, we observed ~ 93–97 % (maximal) relaxation and on the other hand, only ~ 55– 59 % relaxation in aorta in response to ACh in both groups. Notably, comparable eNOS expressions were observed in MAs (Fig. 4D–E) and aorta (Fig. 5D–E) of L-NAME mice which was opposite to other reports (Sheng et al., 2017; Wang et al., 2017). This possibly is likely due to 28 days exposure to L-NAME which, was enough to lower eNOS expression (not significant) in our study. This would suggest that a longer exposure to L-NAME might result in a significant change in eNOS expression. Also, measuring NO bioavailability and phosphorylated -eNOS may reveal further shed light in our study. However, our BP responses in L-NAME hypertensive mice were in agreement with other reports (Berkban et al., 2015; Gao et al., 2011; Nishijima et al., 2014; Paulis et al., 2008). Reduced α -adrenergic receptor-induced responses may occur to compensate for higher BP responses by reducing expression levels of α -1 adrenergic receptors. This claim needs to be investigated in our L-NAME model of hypertension in mice.

Previously, it was reported that genetic deletion of A₁AR attenuated blood pressure responses in L-NAME induced hypertension mice (Gao et al., 2011). These authors showed that the absence of A₁AR reduced ANG II-induced contractions and reactive oxygen species generation in renal afferent arterioles obtained from L-NAME hypertensive mice. Among, all 4 ARs subtype, A₁ and A₃ AR cause contractions of arteries (Hein et al., 2013; Jacobson and Gao, 2006; Mubagwa and Flameng, 2001; Mubagwa et al., 1996; Ohisalo, 1987; Olanrewaju and Mustafa, 2000; Olanrewaju et al., 2000). In aorta, A₁ is the major AR (Kunduri et al., 2013a; Kunduri et al., 2013b; Nayeem et al., 2008; Ponnoth et al., 2012a; Pradhan et al., 2015); whereas in MAs, A₁ is the only AR that regulates contraction (Teng et al., 2013; Yadav et al., 2015). Also, stimulation of A₁AR inhibits Bk channels thereby inhibits relaxation in aortic smooth muscle cells (Kunduri et al., 2013a). Thus, a possible important role of inhibiting relaxation through A₁AR exists in L-NAME hypertensive mice. We investigated this possibility in this study. We investigated whether ARs dependent responses were altered in MAs and aorta of L-NAME hypertensive mice. Our data revealed enhanced A₁AR-induced (with A₁ selective agonist) contractile responses in MAs (Fig. 2A) and aorta (Fig. 3A) of hypertensive mice. In MAs, AR agonist-induced responses were comparable (Fig. 2B). In mouse MAs, A_{2B}AR mediates vasodilation (Teng et al., 2013; Yadav et al., 2015) which were partly dependent on K_{ATP} channel activity (Yadav et al., 2015). In the present report, we found that K_{ATP} channel (pinacidil) dependent relaxation of MAs were significantly higher (Fig. 2C) in hypertensive mice. It is highly likely, that A_{2B}AR expression may have increased in MAs. Thus, AR-induced vascular contraction and relaxation may negate each other thereby producing comparable ARs induced responses in MAs. Contrary, in aorta of hypertensive mice, AR-induced vascular relaxation was reduced whereas higher contraction was observed (Fig. 3B). In mouse aorta, A_{2A} (major) and A_{2B} AR (partly) cause vascular relaxation, while A_{2A}AR dependent vascular relaxation involves

activation of K_{ATP} channels (Ponnoth et al., 2012b; Sharifi-Sanjani et al., 2013; Zhou et al., 2013). In mouse coronary artery, $A_{2A}AR$ induced relaxation was partially mediated by Nox2-derived hydrogen peroxide (Zhou et al., 2015a). In aorta of high salt fed mice, $A_{2A}AR$ induced vascular relaxation involves EETs, PPAR γ , and K_{ATP} channels (Pradhan et al., 2015). In our study, we found that K_{ATP} channel induced responses were comparable in aorta (Fig. 3C). Thus, various ARs dependent relaxation pathways exist in aorta which may be affected in L-NAME induced hypertensive mice contributing towards high BP.

A_1AR -induced contractions in MAs and aorta were mediated by the activity of Cyp4A (Jacobson and Gao, 2006; Sanjani et al., 2011; Yadav et al., 2016; Zhou et al., 2015a). Likely, higher A_1AR -induced contractions involve Cyp4A in L-NAME hypertensive mice. As expected, we found Cyp4A dependent higher contractions in L-NAME hypertensive mice MAs (Fig. 2A). Furthermore, in aorta, Cyp4A was involved in the higher contractile responses in L-NAME hypertensive mice (Fig. 3A). Importantly, in control aorta, the A_1AR -induced contraction was dependent on Cyp4A (Fig. 3A). Thus, our data indicated that Cyp4A mediates A_1AR -induced contractions in MAs and aorta of L-NAME hypertensive mice. In agreement with our functional data, our expression analysis revealed that A_1AR and Cyp4A are higher in L-NAME hypertensive mice MAs (Fig. 4A–C). In aorta, higher A_1AR and comparable Cyp4A were observed in L-NAME hypertensive mice (Fig. 5 A–C). Recently, increase in 20-HETE (a product of Cyp4A activity) levels in the resistance arteries of L-NAME hypertensive rats was reported (Özen et al., 2017). Furthermore, Cyp4A inhibition attenuated the increase in BP in L-NAME hypertensive rats. Notably, Cyp4A inhibition improved vascular relaxation and contraction responses in the conduit and resistance arteries (Özen et al., 2017). Thus, indicating that in our study, Cyp4A downstream of A_1AR may be involved in L-NAME induced hypertensive mice. Also, higher expression and contractions of A_1AR in MAs and aorta contributes towards high blood pressure in L-NAME hypertensive mice.

Conclusion

Our study revealed the important role of A_1AR in L-NAME induced hypertensive mice. We found an enhanced A_1AR induced vascular contractions in MAs and aorta of L-NAME hypertensive. Involvement of Cyp4A, a downstream signaling molecule of A_1AR contraction pathway enhances vascular reactivity significantly in the present study. Importantly, our data revealed that AR-dependent relaxation was inhibited in aorta. Thus, a future study aiming towards investigating changes in the A_{2A} and A_{2B} dependent signaling may unveil the complete role of ARs in L-NAME induced hypertension.

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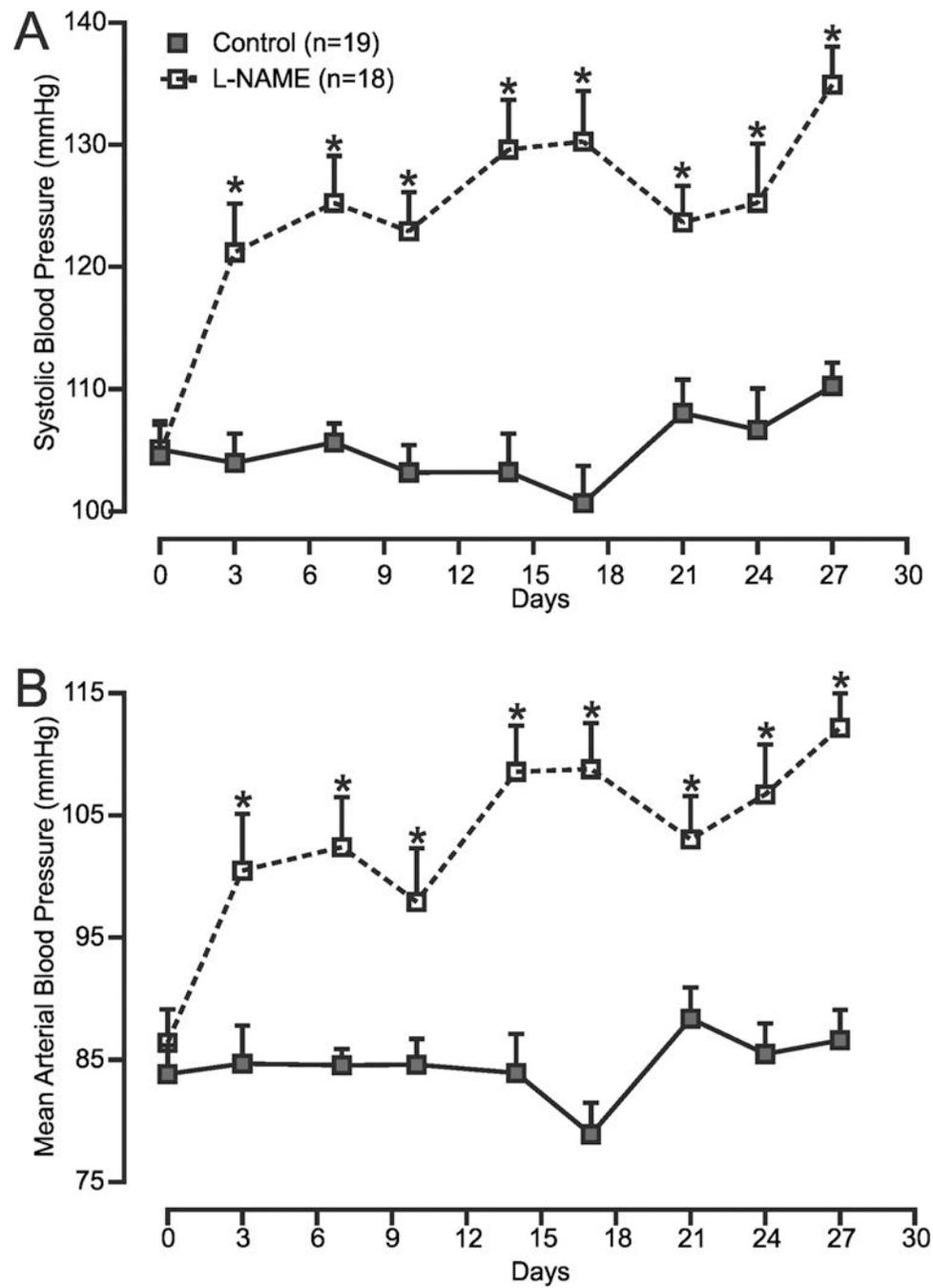


Fig. 1. L-NAME in drinking water increases BP in mice. Shown are (A) SABP and (B) MABP data recorded from day 0–28. * $P < 0.05$ compared to control at respective day BP.

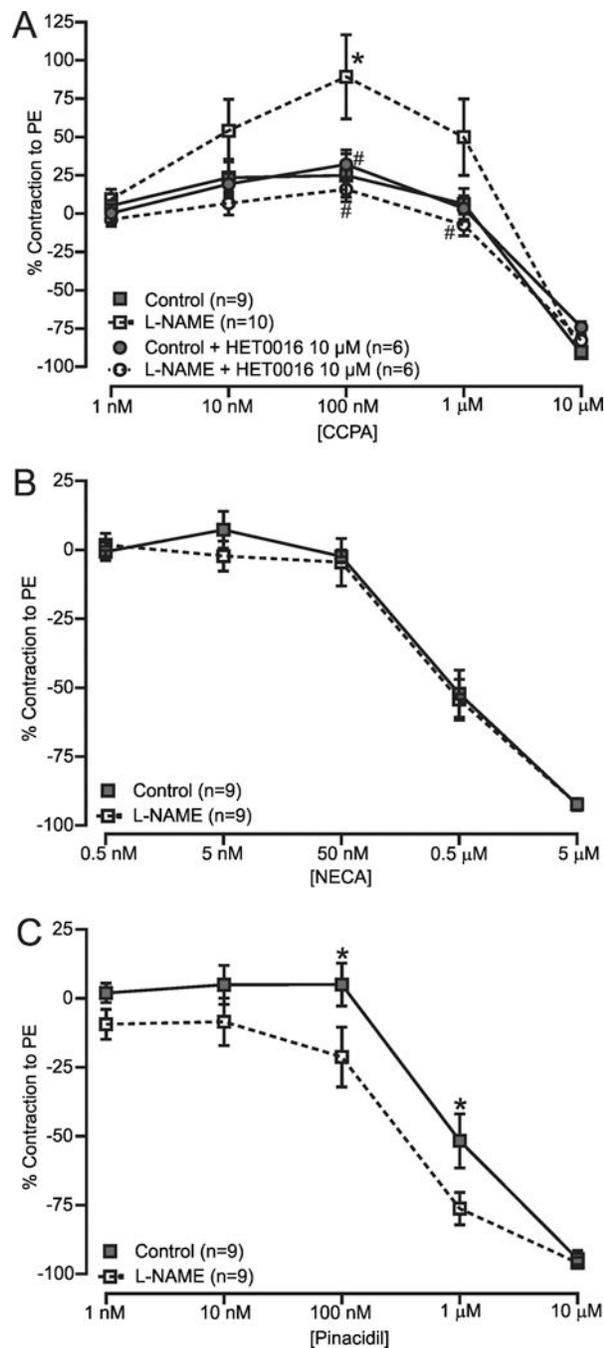


Fig. 2. Higher A_1AR -induced, CYP4A dependent contraction, and enhanced pinacidil induced relaxations were present in MAs of L-NAME mice. (A) CCPA (A_1 selective agonist)-induced contraction response curves (CRCs) and the effect of Cyp4A inhibitor, HET0016 (10 μ M) on CCPA induced CRCs; (B) NECA and (C) Pinacidil induced CRCs in control and L-NAME mice. * indicates $P < 0.05$ compared to control and # denotes $P < 0.05$ compared to L-NAME response at a respective concentration.

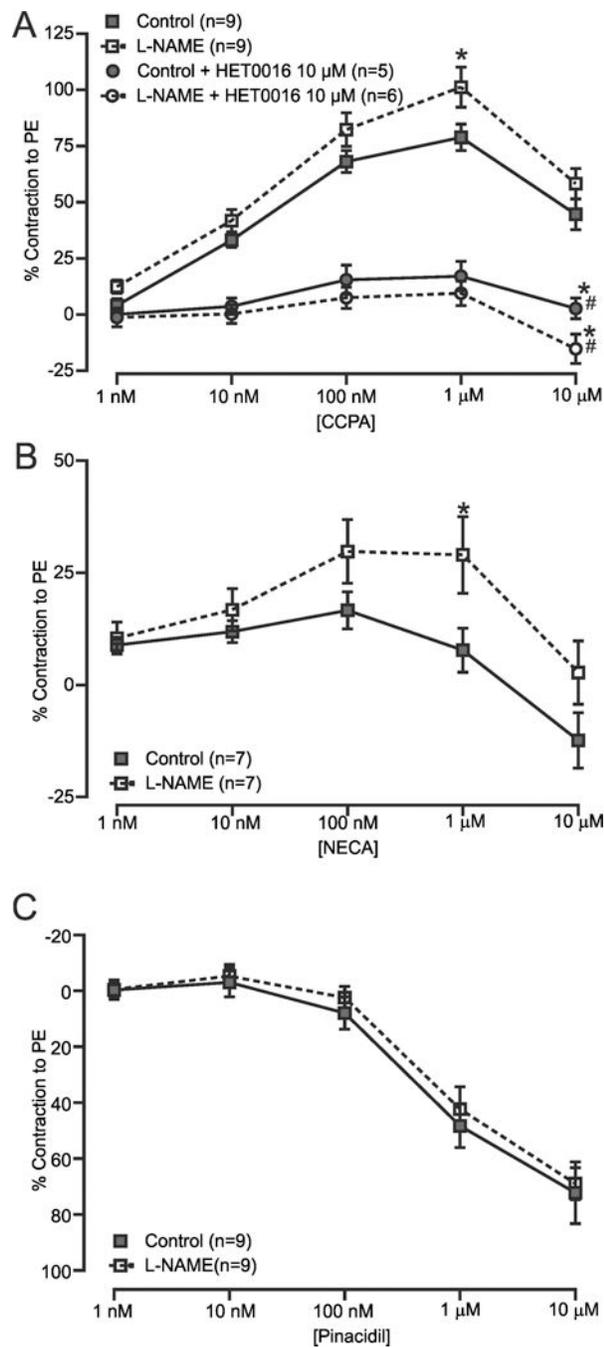


Fig. 3. A_1 AR induced and Cyp4A dependent higher contractions were observed in aorta of L-NAME mice. (A) CCPA and effect of HET0016 on CCPA induced CRCs; (B) NECA; (C) Pinacidil induced CRCs in control and L-NAME mice. * $P < 0.05$ compared to control, # $P < 0.05$ compared to L-NAME.

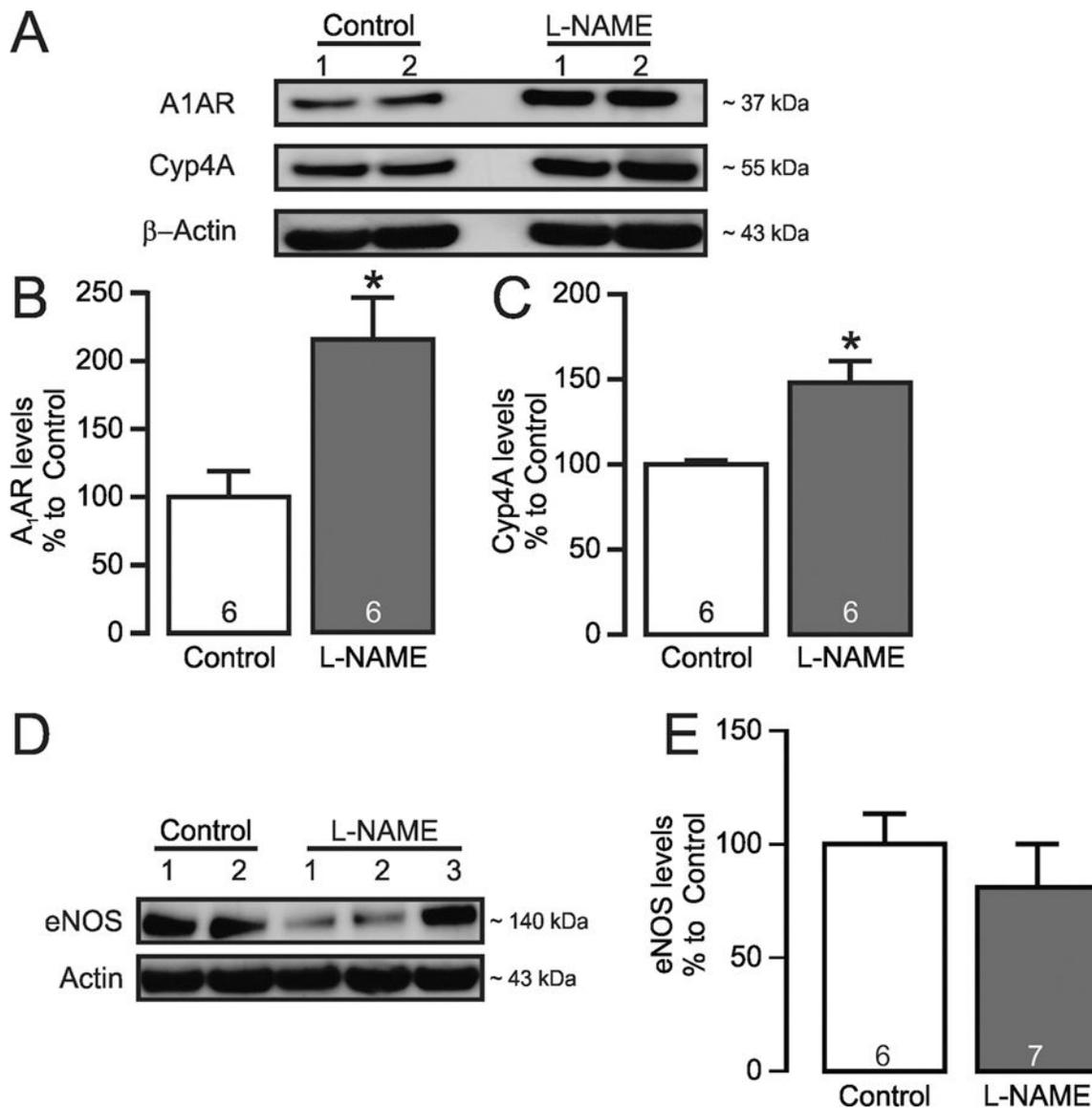


Fig. 4. Expression levels of A₁AR, Cyp4A, and eNOS in MAs of L-NAME hypertensive mice. **(A)** The panel shows representative western blot of A₁AR, Cyp4A, and actin from two different mice. Bar graph summarizes quantification of A₁AR **(B)** and Cyp4A **(C)**. **(D)** Representative blot of eNOS expression from two control and 3 L-NAME mice MAs. **(E)** Quantification of eNOS levels. * P < 0.05 compared to control. The number in the bar graph shows number of animals for the experiment.

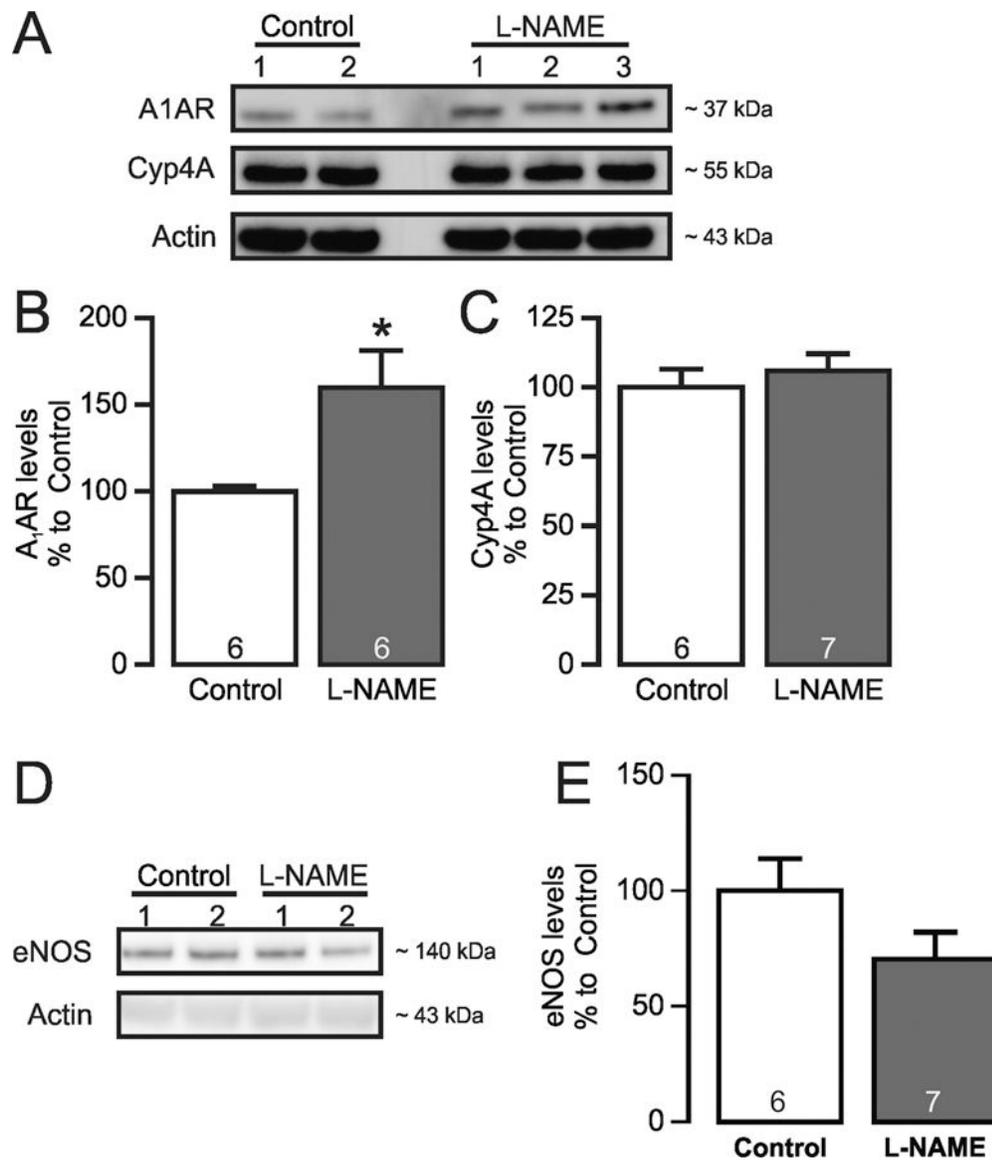


Fig. 5. Expression levels of A₁AR and Cyp4A in aorta of L-NAME hypertensive mice. **(A)** Representative western blot of A₁AR, Cyp4A, and actin from two different mice. Bar graph summarizes quantification of **(B)** A₁AR and **(C)** Cyp4A. **(D)** Representative blot of eNOS expression from aorta of two control and 3 L-NAME mice. **(E)** Quantification of eNOS levels. * P < 0.05 compared to control. The number in the bar graph shows number of animals for the experiment.