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Cardiovascular effects of arginase inhibition in spontaneously hypertensive rats with fully developed hypertension

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Aims Growing evidence suggests that arginase misregulation plays a key role in the pathophysiology of essential hypertension. In the present study, we investigated the potential cardiovascular therapeutic effects of a long-term treatment with an arginase inhibitor in adult spontaneously hypertensive rats (SHR) with fully developed hypertension.

Methods and results Treatment of 25-week-old SHR with the arginase inhibitor N_{ω}-hydroxy-nor-L-arginine (nor-NOHA, 40 mg/day for 10 weeks) sustainably reduced systolic blood pressure (-30 mmHg, $P < 0.05$). The antihypertensive effect of nor-NOHA was associated with changes on mesenteric artery reactivity including the restoration of angiotensin-II-induced contraction and acetylcholine-induced vasodilation to the values of normotensive Wistar Kyoto rats. Both nitric oxide synthase and cyclooxygenase-dependent mechanisms account for the improvement of endothelial function afforded by the arginase inhibitor, which in addition blunted hypertension-induced endothelial arginase I overexpression in mesenteric arteries. Nor-NOHA also prevented the remodelling of aorta as measured by collagen content and media/lumen ratio, and improved the compliance of carotid artery in SHR. Cardiac fibrosis assessed by collagen content of left heart ventricle was reduced by nor-NOHA, with no significant effect on cardiac hypertrophy.

Conclusion Our results report that a long-term treatment with an arginase inhibitor reduced blood pressure, improved vascular function, and reduced cardiac fibrosis in SHR with fully developed hypertension. These data suggest that arginase represents a promising novel target for pharmacological intervention in essential hypertension.

Keywords Arginase • Arteries • Endothelial function • Remodelling • Spontaneously hypertensive rat

1. Introduction

Arginase (EC 3.5.3.1) is a hydrolytic enzyme responsible for converting L-arginine to urea and L-ornithine, the synthesis precursor of polyamines. Mammalian arginases exist in two distinct isoforms (type I and type II) which are encoded by separate genes, have specific subcellular localization and tissue distribution. Arginase I is a cytosolic enzyme located primarily in the liver, whereas arginase II is located within the mitochondrion and is expressed at high levels in the kidneys.[1] However, both arginase isoforms are also expressed by endothelial and vascular smooth muscle cells (VSMC).[2–4] Because nitric oxide synthase (NOS) and arginase use L-arginine as a common substrate, arginase may downregulate nitric oxide (NO) biosynthesis by competing with NOS for L-arginine degradation. Consistent with this hypothesis, NO production has been inversely correlated to arginase activity in vessels at both physiological[4] and pathological conditions such as hypertension,[5] atherosclerosis,[6] diabetes,[7] erectile dysfunction,[8] and aging.[9]

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Endothelial dysfunction and associated impairment in vascular NO production have been largely involved in the pathogenesis of arterial hypertension.[10] Surprisingly, the role of arginase in hypertension is poorly documented. Increased arginase activity/expression was reported in various vascular beds in models of essential or secondary hypertension.[11–14] In addition, upregulation of arginase activity contributes to attenuation of cutaneous vasodilation in hypertensive humans.[15] Recent studies from our laboratory reported that arginase inhibitor prevented the development of hypertension and improved aortic endothelial function via a NO-dependent mechanism when administered to prehypertensive or young adult spontaneously hypertensive rats (SHR).[5,16] From these experimental and clinical data have emerged the concept that arginase inhibition may represent a novel strategy in hypertension. However, the potential antihypertensive effect of arginase inhibition in SHR with fully developed hypertension remains to be investigated. The efficacy of an antihypertensive treatment should not to be solely estimated by the extent of blood pressure (BP) reduction. Above and beyond BP values, it is necessary to monitor the impact of the antihypertensive treatment on hypertension-induced organ damage including vascular and cardiac remodelling as well as arterial compliance.[17] Again, so far the effect of arginase inhibitor on these parameters is unknown.

The present study investigates the potential cardiovascular therapeutic effects of arginase inhibitor in adult SHR with fully developed hypertension. For this purpose, N_ω-hydroxy-nor-L-arginine (nor-NOHA), a selective arginase inhibitor, was daily administered to SHR at 25 weeks age for 10 weeks. The impact of treatment was studied on systolic BP, reactivity of resistance vessels to vasodilating and vasoconstrictive drugs, vascular and cardiac remodelling and artery compliance. Wistar Kyoto rats (WKY) rats were evaluated in parallel in order to investigate to what extent nor-NOHA-treated SHR can recover in relation to control values.

2. Methods

2.1 Animals and treatment

Twenty-three male WKY rats and 42 SHR were purchased from Charles River (L'Arbresle, France). Animals were kept under a 12–12 h light:dark cycle and allowed free access to food and water. The investigation was approved by the local Ethical Committee and conforms to the Guide for the Care and Use of Laboratory Animal published by the US National Institutes of Health (NIH publication No. 85–23, revised 1996).

At 25 weeks at age, SHR were randomly divided into two groups: SHR ($n = 21$) and nor-NOHA-treated SHR ($n = 21$). Nor-NOHA (Bachem, France) was dissolved in 0.9% NaCl and administered once a day (40 mg/kg, ip) for 10 weeks. Control SHR received an equal volume of saline daily. A group of untreated age-matched WKY rats was used as controls ($n = 23$). Systolic blood pressure (SBP) was measured once a week in conscious rats by using the indirect tail-cuff method (BP2000, Visitech System, Apex, NC, USA). At the end of the treatment period, rats were anaesthetized (sodium pentobarbital, 60 mg/kg, ip) in order to collect blood and various organs. Arginase activity was determined in liver and kidney from the measurement of urea production according to the method of Corraliza *et al.*,[18] as previously described.[5] Glucose levels were measured in total blood using a blood glucose meter (Glucomen Glycó, A. Menarini diagnostics, Antony, France).

2.2 Vascular function

A third order branch of the mesenteric artery was isolated and mounted on a pressurized myograph as described previously.[16] After precontraction with 10^{-5} M phenylephrine (PE), endothelium-dependent and -independent relaxation was assessed with acetylcholine (ACh, 10^{-10} to 10^{-4} M) and sodium nitroprusside (SNP, 10^{-10} to 10^{-4} M). To study the role of NO, eicosanoids, and endothelium-derived hyperpolarizing factor (EDHF) in ACh-dependent relaxation, vessels were pretreated (20 min) with the NOS inhibitor N_ω-nitro-L-arginine methyl ester (L-NAME, 10^{-4} M), the cyclooxygenase (COX) inhibitor indomethacin (10^{-5} M), the Ca^{2+}-dependent K^+ channel inhibitors apamin (10^{-7} M) and charybdotoxin (10^{-7} M), respectively. Moreover, the response to PE (10^{-10} to 10^{-4} M), angiotensin-II (ANG-II, 10^{-10} to 10^{-4} M), and endothelin-1 (ET-1, 10^{-10} to 10^{-4} M) was investigated on mesenteric arteries with intact endothelium as well as in preparations in which endothelium was removed by perfusion with the detergent 3-[(3-cholamidopropyl) dimethylammonio]-1-propane sulfonate (0.5%, 45 s). Complete endothelial removal was verified by the absence in vasorelaxation capacity in response to ACh in PE-preconstricted vessels.

2.3 Vascular compliance

The compliance was determined from the biomechanical analysis of the left carotid (LCC) artery as previously described in detail.[19,20] The LCC artery was exposed in rats and mounted in our extension-inflation device described elsewhere.[19,20] The LCC artery was stretched to the individual *in vivo* length for each rat. The artery was maintained in Krebs–Ringer solution at 37°C and at a pH of 7.4 (continuously bubbled with standard gas: O_2–CO_2 95–5%). The segment was then inflated with the constant rate of ~5 mmHg/s. External diameter and intraluminal pressure were measured. Arteries were preconditioned by means of inflation–deflation loops (pressure range: 0–230 mmHg for WKY and 0–270 mmHg for SHR and nor-NOHA samples) until the pressure–diameter (P–D) curve became reproducible. The ascending limb of the last stable loop was used for data analysis under normal conditions (normal VSMC tone). Then, KCl was added to the bath to reach the final concentration of 100 mM, while the artery was maintained at 100 mmHg. After 10 min, pressure was lowered to 0 mmHg and raised to 230 mmHg for WKY arteries and 270 for SHR and nor-NOHA group, yielding the P–D curve under maximal contraction (active VSMC state). Having the P–D sets of data and measuring the zero load state geometry of the artery (inner and outer diameter), the area compliance is determined as the slope of the cross-sectional area (CSA)–pressure curve, $C(P) = dA/dP$, where A is the lumen CSA and P the interluminal pressure as previously described.[19,20]

2.4 Vascular remodelling

Remodelling of mesenteric arteries was assessed from the Media/Lumen ratio (M/L) and media CSA as described previously.[16] CSA was calculated from the internal (D_i) and external (D_e) diameters when the artery was at 75 mmHg pressure according to the formula: $CSA = \pi\ (D_e^2 - D_i^2)/4$. Remodelling of aorta was assessed from the following parameters: (i) the M/L ratio measured in paraffin-embedded tissue sections of aorta after Masson's trichromic staining, (ii) the collagen type I content measured in Masson's trichromic-stained sections and the elastin content measured after orcein staining, (iii) the extracellular matrix metalloproteinases (MMPs) activity measured in aortic homogenates by using the gelatin zymography method, as described below.

2.5 Expression of vascular enzymes

Frozen 8 μm sections of the mesenteric arteries were used for the detection of arginases and COX. Slides were incubated with and without (negative control) a primary antibody against rabbit anti-arginase I, rabbit anti-arginase II, rabbit anti-COX-1, and rabbit anti-COX-2 and then exposed to a fluorescent secondary antibody (IgG Alexa red conjugated antibody, BD Biosciences). Finally, sections were mounted with DAPI-containing mounting medium (Vectashield) and slides were analysed by using a confocal microscope (Leica). The arterial expression of the enzymes was quantified based on the intensity of the fluorescent staining

subtracted by background intensity of negative control. Mean fluorescence intensity was obtained by dividing fluorescent intensity by the area of the artery and reported as arbitrary units. Details of the method are available in Supplementary Material.

2.6 Cardiac remodelling

Cardiac remodelling was assessed from different parameters including the heart/body weight ratio (as an index of hypertrophy), the collagen I and III content of the left ventricle (LV) after picrosirius red staining of paraffin-embedded tissue, and MMPs activity of LV homogenates using the gelatin zymography method, as described below.

2.7 Histological evaluation of the heart and aorta

Tissues were fixed in Bouin's solution and embedded in paraplast. Transversally cut sections (5 μm) were stained with Masson's trichromic method for determination of the collagen type I content, and with picrosirius red (0.1% solution in saturated aqueous picric acid) for determination of the collagen type I and III content. In addition, cross-section of aortic artery was stained with orcein to determine elastin content. Collagen or elastin content was measured with a semi-automated image analysis software (AnalySIS 3.1, Soft Imaging System). The density of collagen or elastin was averaged in 16 fields per section at a magnification of ×200 and expressed as percentage of the total area within a field.

2.8 Gelatin zymography

Gelatinolytic activity of aortic and cardiac tissue homogenates was analysed by electrophoresis in the presence of 8% SDS–polyacrylamide gels containing 1 mg/mL gelatin. Samples were applied to the gel in a sample buffer containing 2.5% SDS but lacking β-mercaptoethanol. A zymography standard (Chemicon International, Temecula, CA, USA) containing pro- and active forms of MMP-2 and MMP-9 was used as positive control. After electrophoresis, gels were incubated at 37°C in buffer (100 mM Tris–HCl, pH 7.4, 100 mM NaCl, 5 mM $CaCl_2$, 5 μM $ZnCl_2$, and 0.02% BRIJ-35). Subsequently, gels were stained with Coomassie brilliant blue G-250 for 1 h and then destained appropriately with a solution of acetic acid/ethanol/water (1/2/7). MMP activity was quantified by measuring optical density with an image analyser (Gel Doc XR, Bio-Rad, Hercules, USA) and expressed as the ratio of density of the sample related to the density of the MMP control.

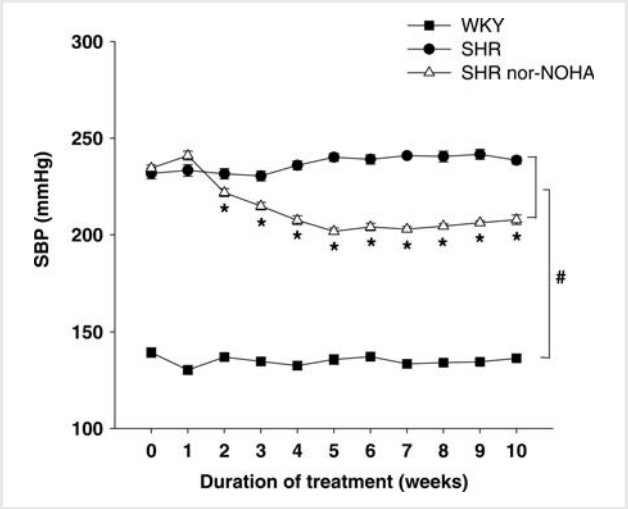

Figure 1 Effect of nor-NOHA treatment on systolic blood pressure (SBP). Twenty-five-week-old SHR were treated with nor-NOHA (40 mg/kg/day, ip) or with vehicle for 10 weeks. SBP was measured by the tail-cuff method before and once a week after the beginning of the treatment. Values are means ± SEM from 21 to 23 rats per group. $^{\#}P < 0.05$ vs. WKY rats, $^{*}P < 0.05$ vs. untreated SHR.

2.9 Statistical analysis

Values are given as means ± SEM. The pD_2 values (calculated as the negative logarithm of the molar concentration that produced 50% of the maximal effect) and Max values (values of maximal relaxation, E_{max}) were determined by fitting the original dose–response curves using Sigma Plot Software and compared by one-way analysis of variance (ANOVA) followed by Bonferroni's test. Dose–response relationships were analysed using two-way ANOVA followed by Bonferroni's test. $P < 0.05$ was considered statistically significant.

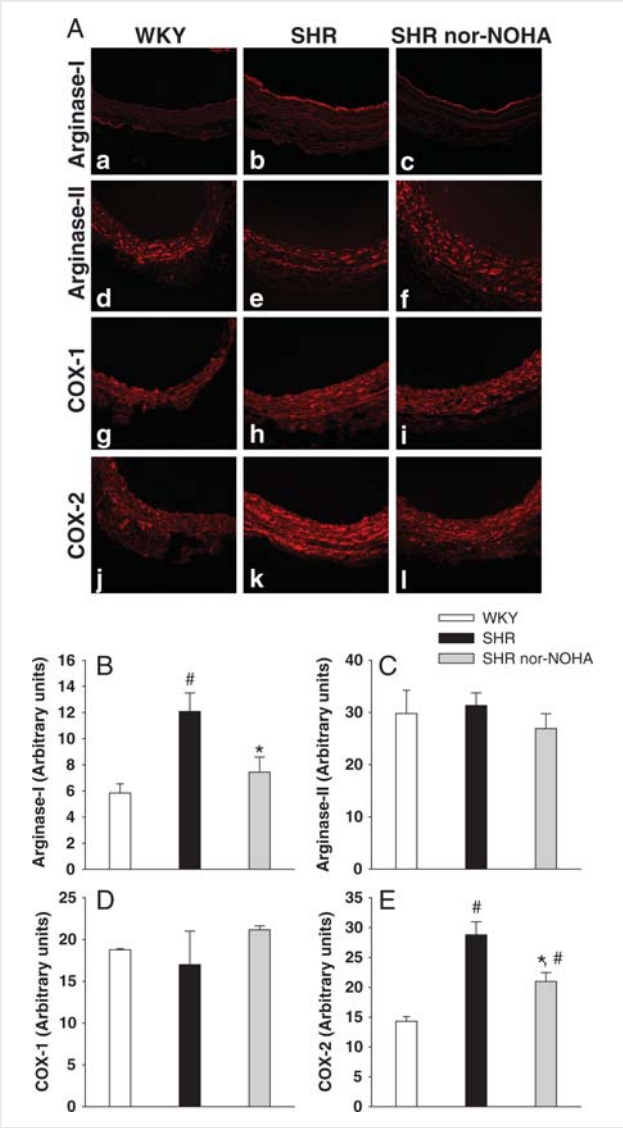

Figure 2 Arginases and COX expression in mesenteric arteries. (*A*) Representative figures (×40) for arginase I (a–c), arginase II (d–f), COX-1 (g–i), and COX-2 (h–j) expression in mesenteric arteries from WKY rats, SHR, and nor-NOHA-treated SHR. (*B–E*) Histograms for quantification of arginase I (*B*), arginase II (*C*), COX-1 (*D*), and COX-2 (*E*) in the different experimental groups. Results are expressed as means ± SEM from 4 to 8 vessels. $^{\#}P < 0.05$ vs. WKY rats, $^{*}P < 0.05$ vs. untreated SHR.

3. Results

3.1 Effects of nor-NOHA on physiological parameters and arginase activity

As shown in *Figure 1*, nor-NOHA significantly decreased SBP in SHR. The antihypertensive effect began from 2 weeks of treatment and reached −30 mmHg after 5 weeks of treatment when compared with untreated SHR ($P < 0.05$). The prolongation of the treatment did not induce further changes in SBP. In addition, untreated SHR and treated SHR exhibited similar blood glucose levels (1.01 ± 0.05 vs. 1.03 ± 0.05 g/L, respectively), heart rates (434 ± 4 vs. 435 ± 6 bpm, respectively), and body weights (381 ± 5 vs. 374 ± 4 g, respectively) (data not shown). Finally, nor-NOHA administration did inhibit both arginase isoforms as evidenced by lower arginase activity in liver (arginase I, −41%, $P < 0.001$) and kidney (arginase II, −40%, $P < 0.001$) in treated SHR than in untreated SHR (data not shown).

3.2 Effects of nor-NOHA on arginase and Cox vascular expression

Results are summarized in *Figure 2*. Genetic hypertension was associated with a two-fold increase in the expression of arginase I in mesenteric arteries ($P < 0.05$, *Figure 2B*), whereas arginase II expression was unchanged (*Figure 2C*). Likewise, SHR exhibited a two-fold increase in expression of COX-2 ($P < 0.05$, *Figure 2E*) but not of COX-1 (*Figure 2D*), as previously described.[21,22] Hypertension-induced upregulation of these enzymes occurred in endothelium for arginase I (*Figure 2A* and *B*) and in both endothelium and VSMC for COX-2 (*Figure 2J* and *K*). Nor-NOHA decreased the expression of arginase I (*Figure 2B* and *C*) and COX-2 (*Figure 2E* and *L*) by 38% ($P < 0.05$) and 27% ($P < 0.05$), respectively.

3.3 Effects of nor-NOHA on vascular function

The results showed that nor-NOHA fully restored the response of mesenteric arteries to ACh (*Figure 3A*) and ANG-II (*Figure 3B*) to that of WKY rats, whereas it failed to ameliorate the response to PE (*Figure 3C*) or ET-1 (*Figure 3D*). The removal of the endothelium did not modify dose–response curves to any vasoconstrictive drugs (data not shown). Additionally, no difference in SNP-induced vasodilation was observed among WKY rats, treated SHR, and untreated SHR (data not shown). In order to dissect the mechanisms involved in the effect of nor-NOHA on ACh-induced vasodilation, the dose–response curve of ACh was studied in the presence of L-NAME, indomethacin, or apamin + charybdotoxin (*Figure 4*).

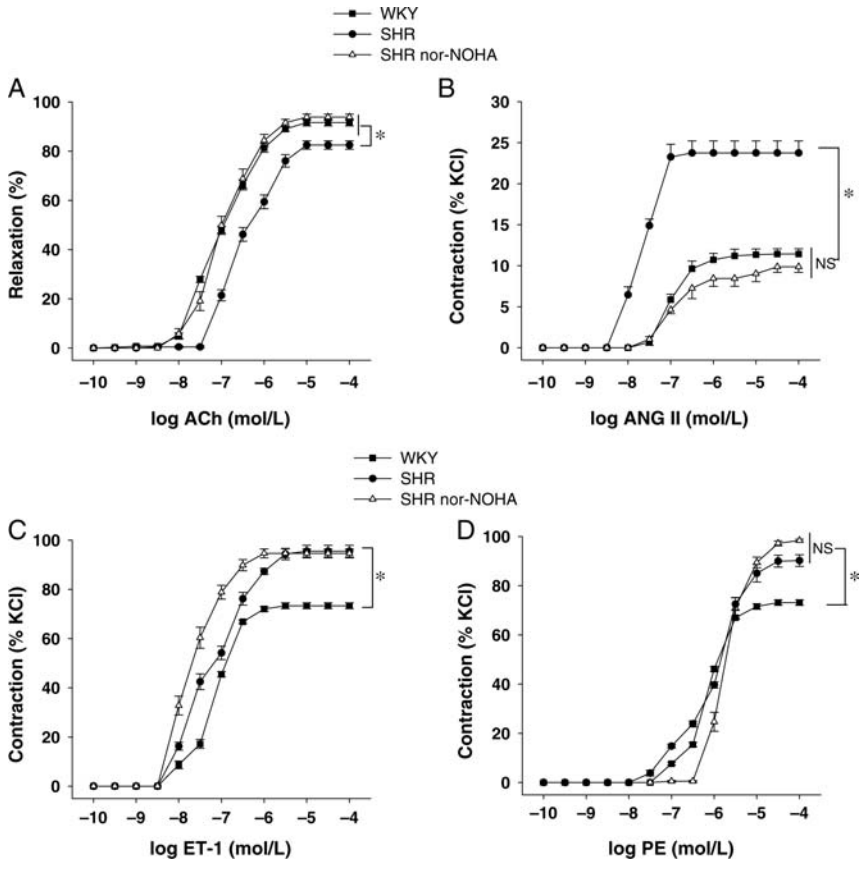

Figure 3 Effect of nor-NOHA on vascular reactivity. Experiments were conducted on the third order branch of the mesenteric artery isolated from WKY rats, SHR, and treated SHR. (*A*) Cumulative concentration curves with acetylcholine (ACh). (*B–D*) Cumulative concentration curves with (*B*) angiotensin-II (ANG-II), (*C*) endothelin-I (ET-1), and (*D*) phenylephrine (PE). The results are expressed as a percentage of the maximal constrictive response to KCl 100 mM. Results are expressed as means ± SEM from eight vessels. *$P < 0.05$.

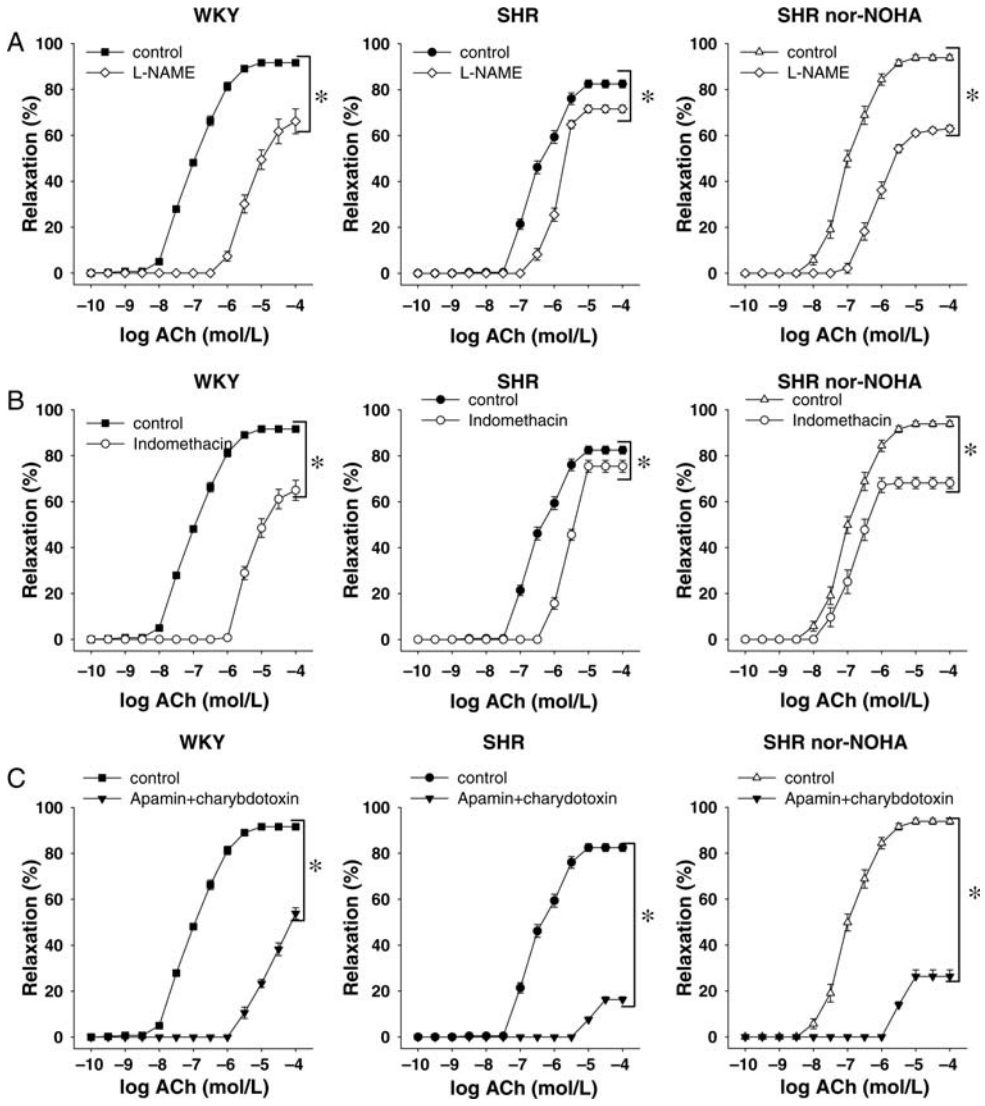

Figure 4 Effect of L-NAME, indomethacin, apamin, and charybdotoxin on the mesenteric artery response to ACh. Experiments were conducted on the third order branch of the mesenteric artery isolated from WKY rats, SHR and treated SHR. The cumulative concentration curves of ACh were performed in the presence or not of (*A*) L-NAME 10^{-4} M, (*B*) indomethacin 10^{-5} M, (*C*) Apamin 10^{-7} M, and charybdotoxin 10^{-7} M. Results are expressed as means ± SEM from eight vessels. $^{*}P < 0.05$.

Moreover, we calculated the E_{max} of ACh with or without the inhibitor and the inhibitory effect of each inhibitor was expressed as the percentage of reduction of E_{max} of ACh alone, as shown in *Table 1*. Such kind of analysis allowed us to statistically compare WKY rats, SHR, and nor-NOHA-SHR with respect to the contribution of NO, COX products, and EDHF in ACh-induced vasodilation. As shown in *Figure 4*, L-NAME, indomethacin and Apamin + charybdotoxin significantly decreased the effect of ACh in all groups. However, the data demonstrate that nor-NOHA significantly improved the NOS- and COX-dependent components of ACh vasodilation in SHR. Indeed, as shown in *Table 1*, the inhibitory effects of L-NAME and indomethacin on ACh-induced maximal vasodilation were greater in treated SHR when compared with untreated SHR and were not different from that of WKY rats. In contrast, the contribution of EDHF in the effect of ACh was not modified by nor-NOHA (*Figure 4C* and *Table 1*).

Table 1 Inhibitory effect of L-NAME, indomethacin, apamin plus charybdotoxin on the maximal ACh-induced vasodilation in mesenteric arteries

	WKY (%)	SHR (%)	SHR nor-NOHA (%)
L-NAME 10^{-4} M	−28 ± 5	−13 ± 2$^{\#}$	−29 ± 2*
Indomethacin 10^{-5} M	−30 ± 4	−7 ± 3$^{\#}$	−27 ± 3*
Apamin (10^{-7} M) + charybdotoxin (10^{-7} M)	−30 ± 5	−80 ± 2$^{\#}$	−72 ± 3$^{\#}$

The effects of drugs are expressed as the percentage of inhibition of the maximal response to ACh 10^{-4} M. Values are means ± SEM from eight vessels.
$^{\#}P < 0.05$ vs. WKY.
$^{*}P < 0.05$ vs. SHR.

3.4 Effects of nor-NOHA on vascular and cardiac remodelling

The effects of arginase inhibition on hypertension-induced remodelling of vessels and heart are shown in *Table 2*. Nor-NOHA prevented remodelling of aorta but not mesenteric arteries. Indeed, aorta media thickness, M/L ratio, and collagen type I content were significantly lower in treated SHR than in untreated SHR (−32, −25, −17%, respectively, $P < 0.05$, *Table 2*, see Supplementary material online, *Figure S1*). However, nor-NOHA did not modify hypertension-induced increase in pro- and native MMP-2 and MMP-9 activities (*Figure 5*), indicating that the decrease in collagen content afforded by arginase inhibition does not involve collagen degradation. Nor-NOHA did not significantly reduce hypertension-induced cardiac hypertrophy (*Table 2*). However, it reduced (−53%, $P < 0.05$, *Table 2*, see Supplementary material online, *Figure S1*) the collagen type I and III deposition by a mechanism independent on MMPs activity as pro-MMP-2 and active MMP-2 were not different between WKY rats, SHR, and nor-NOHA-treated SHR (see Supplementary material online, *Figure S2*).

3.5 Effects of nor-NOHA on vascular compliance

LCC artery compliance of each group have been calculated under normal, maximally active (KCl) and passive tone (SNP) and at their corresponding mean physiological pressures (135 mmHg for WKY rats, 235 mmHg for untreated SHR and 200 mmHg for treated SHR). As shown in *Figure 6A*, hypertension-associated decrease in arterial compliance was significantly improved by nor-NOHA during normal ($P < 0.01$), active ($P < 0.001$), and passive tone ($P < 0.001$). Furthermore, at the pressure of 200 mmHg (i.e. the physiological pressure for the treated SHR), nor-NOHA still improved arterial compliance in KCl-contracted arteries ($P < 0.005$) and SNP-treated arteries ($P < 0.05$) when compared with untreated SHR (*Figure 6B*). These data indicate that the effects of nor-NOHA on arterial compliance were not only due to pressure reduction but rather reflected intrinsic changes in the vascular tissue.

4. Discussion

Increased vascular arginase expression has been previously reported in animal models of hypertension. Secondary hypertension is associated with the upregulation of both arginase isoforms in gracilis muscle arterioles from Dahl-salt sensitive rats,[12] whereas only the isoform I is overexpressed in coronary arterioles from pigs with aortic coarctation.[13] In genetic hypertension, we recently reported an increase in the expression of both arginase isoforms in aorta of SHR from the age of 5−26 weeks.[14] The present study reports a selective upregulation of the isoform I in mesenteric arteries of 35-week-old SHR, suggesting that the misregulation of the arginase pathway is vessel and/or age-dependent in genetic hypertension. Moreover, our results revealed that the decrease in arginase I expression in nor-NOHA-treated SHR is associated to a decrease in SBP. This result reinforces our previous findings that hypertension, on its own, contributes to upregulate vascular arginase.[14] Importantly, the long-lasting and steady antihypertensive effects of the long-term treatment with nor-NOHA reported here is consistent with the idea that prolonged arginase inhibition does not induce a compensatory upregulation of the enzyme.

Both endothelial dysfunction and abnormalities in VSMC reactivity are known to contribute to increased vascular resistances in essential hypertension.[23−26] The present study provides arguments that arginase inhibition controls vascular tone of resistance vessels not only through increased NO production at the endothelial level as previously described[27] but also through a decreased VSMC reactivity to ANG-II. Moreover, our results indicate that besides increased NO production, COX-dependent mechanisms are involved in the improvement of ACh-induced vasodilation by nor-NOHA. Evidence of decreased vascular COX-2 expression by nor-NOHA strongly suggests that the production of vasoconstrictor eicosanoids by COX-2 is reduced after arginase inhibition. However, increased production of vasodilating eicosanoids cannot be excluded since endothelial COX activity is stimulated by NO.[28] Further studies are needed to elucidate the intimate mechanisms involved in the

Table 2 Effects of nor-NOHA on vascular and cardiac remodelling

	WKY	SHR	SHR nor-NOHA
Mesenteric artery			
Media thickness (μm)	18.8 ± 0.5	26.8 ± 0.9*	26.2 ± 0.9*
Lumen (μm)	282.7 ± 5.6	282.7 ± 4.8	255.8 ± 8.7
M/L ratio (%)	6.7 ± 0.2	9.5 ± 0.3*	10.4 ± 0.4*
Media cross-sectional area (μm^2)	8638.1 ± 321.5	12492.1 ± 518.1*	11203.0 ± 625.3*
Aorta			
Media thickness (μm)	53.1 ± 1.4	97.6 ± 0.9*	66.2 ± 2.3*#
Lumen (μm)	1675.1 ± 26.9	1778.0 ± 29.7	1601.1 ± 44.6
M/L ratio (%)	3.2 ± 0.1	5.5 ± 0.3*	4.1 ± 0.3*#
Collagen type I (% of area)	22.1 ± 2.4	36.9 ± 3.8*	30.7 ± 3.0*#
Elastin (% of area)	20.8 ± 1.9	23.7 ± 2.4	23.3 ± 0.9
Heart			
Heart/body weight ratio (%)	23.2 ± 0.3	36.8 ± 0.6*	38.3 ± 0.5*
Left ventricle intersticial collagen type I and III (% of area)	4.4 ± 0.4	11.3 ± 1.5*	5.3 ± 0.4*#

Values are means ± SEM ($n = 8$).
*$P < 0.05$ different from WKY rats.
#$P < 0.05$ different from untreated SHR.

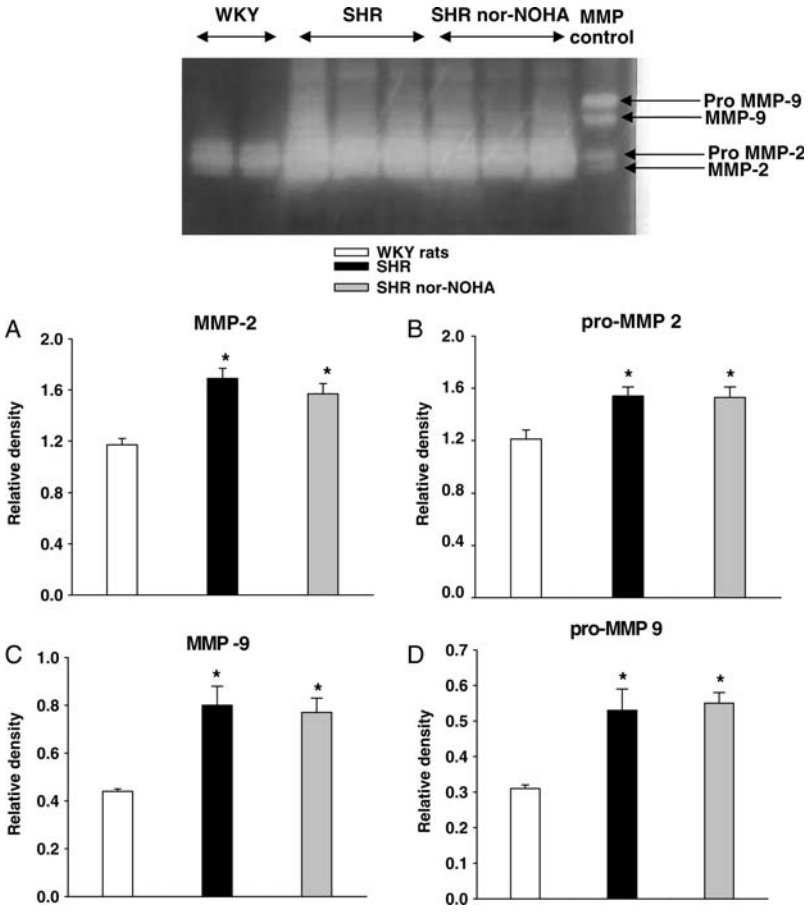

Figure 5 Effect of nor-NOHA on MMP activity in aortas. MMP-2 and MMP-9 activities were determined by gelatin zymography of aortic tissue from WKY rats, SHR and treated SHR. The upper panel shows a representative zymogram of samples and of the MMP control (last right lane), and the lower panels illustrate the densitometric analysis of lytic bands (expressed as the ratio of density of the sample related to the density of the MMP control) of MMP-2 (*A*), pro-MMP-2 (*B*), MMP-9 (*C*), and pro-MMP-9 (*D*). Values are means ± SEM ($n = 8$). *$P < 0.05$ vs. WKY rats.

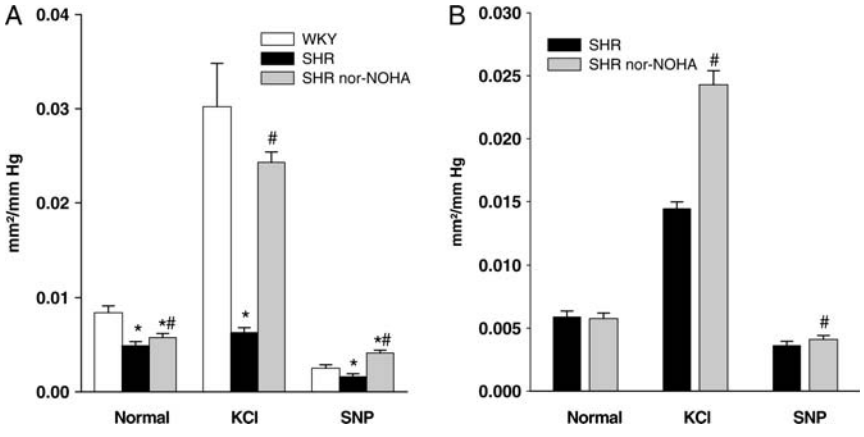

Figure 6 Effect of nor-NOHA on carotid artery compliance. Arterial compliance was determined in carotid arteries from WKY rats, SHR and treated SHR, as described in Methods. The measurements were made (*A*) under corresponding physiological pressure for each group and (*B*) at the nor-NOHA-treated group pressure (200 mmHg), under normal resting (normal), totally contracted (KCl), and totally dilated (SNP) state of vascular smooth muscle cells. Values are means ± SEM from 6–8 vessels. *$P < 0.05$ vs. WKY rats, #$P < 0.05$ vs. untreated SHR.

crosstalk between arginase, ANG-II, and COX pathways in hypertension.

Vascular remodelling of conduit and resistance vessels is well identified as a contributing factor to the hypertensive process.[29] In fact, structural alterations at arterial wall are not only a normal adaptation to raised BP, but also participate to the persistence of the increased BP. Our data provide the first evidence that arginase inhibition can prevent aortic remodelling observed in essential hypertension. Considering that vascular remodelling is an adaptative response to the BP elevation,[30] the antihypertrophic effect of nor-NOHA may be related to the long-lasting decrease in BP afforded by the treatment. However, based on previous studies using different antihypertensive drugs, the reduction in BP by itself appears to be not sufficient to reduce cardiovascular remodelling.[31] Besides the synthesis of urea, arginase is also involved in the production of ornithine, the precursor of polyamines and proline.[1] Thus, nor-NOHA might exert antihypertrophic effects through a decrease in the synthesis of polyamines, compounds largely involved in cell growth/proliferation.[32,33] Indeed, results from *in vitro* experiments showed that polyamine synthesis is increased after stable transfection of arginase cDNA in cultured endothelial cells.[34] Likewise, the reduction of collagen deposition in aorta afforded by nor-NOHA might derive from a decrease in the availability of proline, the precursor of collagen synthesis. In agreement, decreased collagen synthesis rather than increased collagen catabolism is likely involved in the reduction of collagen deposition as evidenced by the lack of effect of arginase inhibition on aortic MMPs activity. However, the benefit of arginase inhibition on aortic remodelling may be in part mediated by a reduction of the VSMCs growth since arginase is essential for cultured rat aortic VSMC to enter in the cell cycle.[35] Of note, nor-NOHA prevented remodelling of aorta but not of mesenteric arteries, suggesting that the mechanisms underlying vascular remodelling might be vessel dependent.[36] Importantly, the lack of impact of nor-NOHA on remodelling of small vessels does not question its potential lowering effect on diastolic BP which is determined by peripheral vascular resistance. Indeed, vascular resistance depends on vascular structure but also on vascular tone[29,37] which is improved after arginase inhibition. Finally, our results also report decreased collagen deposition in heart without accompanying changes in MMPs activity after nor-NOHA treatment. This data may open new perspectives on the regulation of cardiac collagen synthesis by arginases after hypertension.

Clinical studies demonstrated that hypertension is associated with stiffening of conduit arteries leading to decreased arterial compliance, which is now recognized as an important and independent cardiovascular risk factor.[38] Conduit vessel stiffness is, in part, regulated by the smooth muscle tone (which is influenced by circulating and endothelium-derived vasoactive mediators including NO and ANG-II) and extracellular matrix.[39] In agreement with previous studies,[40,41] the present results showed that arterial compliance is reduced in untreated SHR when compared with WKY rats. The new finding of our study is that nor-NOHA treatment dramatically increases the arterial compliance of SHR. Of note, a recent study showed that the arginase inhibitor *S*-(2-boronoethyl)-l-cysteine restored arterial compliance in atherosclerotic apolipoprotein-E deficient mice.[6] Taken together, these data suggest that arginase is a new potential target to reduce arterial stiffness.

In conclusion, our results demonstrate that arginase inhibition in SHR with fully developed hypertension reduced systolic BP and target organ damage including artery remodelling, cardiac fibrosis, and changes in vascular compliance. These results suggest that arginase is a promising target to reduce BP and to improve cardiovascular function in patients with essential hypertension. Arginase inhibition might also be useful for reducing cardiovascular risk in hypertensive patient since a large meta-analysis of trials in hypertension reported that antihypertensive drug treatment improves cardiovascular outcome mainly through lowering of SBP.[6] Interestingly, recent clinical studies confirmed the critical role of arginase in the control of BP. More precisely, plasma arginase activity is high in hypertensive pre-eclamptic women and correlates with plasma NO and BP levels.[42] Additionally, BP level, risk of myocardial infarction and common intima-media thickness are dependent on arginase I polymorphisms.[43,44]

Supplementary material

Supplementary material is available at *Cardiovascular Research* online.

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