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Astaxanthin-enriched-diet reduces blood pressure and improves cardiovascular parameters in spontaneously hypertensive rats

José Monroy-Ruiz [a], María-Ángeles Sevilla [b,*], Rosalía Carrón [b], María-José Montero [b]

[a] Departamento de Salud, Universidad Iberoamericana, Prolongación Paseo de la Reforma 880, Ciudad de México, Mexico
[b] Departamento de Fisiología y Farmacología, Facultad de Farmacia, Universidad de Salamanca, Campus Miguel de Unamuno, 37007 Salamanca, Spain

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ABSTRACT

The aim of this study was to investigate the effects of astaxanthin-enriched diet on blood pressure, cardiac hypertrophy, both vascular structure and function and superoxide ($^{\bullet}O_2^-$) production in spontaneously hypertensive rats (SHR).

Twelve-week-old SHR were treated for 8 weeks with an astaxanthin-enriched diet (75 or 200 mg/kg body weight per day). Systolic blood pressure was monitorized periodically during the study by the tail cuff method. At the end of the study animals were sacrificed and heart, kidneys and aorta were removed. Left ventricular weight/body weight ratio was used as left ventricular hypertrophy index (LVH). Vascular function and structure were studied in conductance (aortic rings) and resistance (renal vascular bed) arteries. Also $^{\bullet}O_2^-$ production was evaluated by lucigenin-enhanced chemiluminescence.

Systolic blood pressure was lower in astaxanthin-treated groups than the control group from the first week of treatment, and LVH was significantly reduced. Astaxanthin improved endothelial function on resistance arteries, but had no effect on aorta. These effects were accompanied by a decrease in oxidative stress and improvements in NO bioavailability. Taken together, these results show that diet supplemented with astaxanthin has beneficial effects on hypertension, by decreasing blood pressure values, improving cardiovascular remodeling and oxidative stress.

1. Introduction

In the last years, our understanding of oxidative stress and its role in vascular disease has increased significantly. It is known the excess of reactive oxygen species (ROS) is associated to inflammation, growth and vasoconstriction contributing to vascular injury in many cardiovascular diseases, such as hypertension, hyperlipidemia, and diabetes [1–5].

ROS play an important pathophysiological role in hypertension. Models such as spontaneously hypertensive rat (SHR), SHR-stroke-prone [6,7], rats with aortic banding-induced or renovascular hypertension (two kidney one clip model) [8,9] and also in rats with deoxycorticosterone acetate-salt induced hypertension [10]. These all exhibit enhanced NAD(P)H oxidase-mediated superoxide anion ($^{\bullet}O_2^-$) generation and increased expression of NAD(P)H oxidase subunits, specifically p22phox and p47phox, and increased activity of the enzyme [11]. Treatment with antioxidants or agents that inhibit NAD(P)H oxidase-driven generation of ROS reduces, and may even prevent, blood pressure elevation in hypertensive animals. This role for NADPH oxidases has been established using genetically modified mice. In transgenic mice, overexpression of p22phox in vascular smooth muscle cells (VSMC) potentiates angiotensin II-induced aortic hypertrophy and hypertension [12]. Mice that lack Nox1 (a subunit of NAD(P)H oxidase) have a blunted pressor response to angiotensin II and increased vasodilation in response to acetylcholine (ACh) [13].

Several studies have shown beneficial effects of treatments with antioxidants such as allopurinol and hydrosoluble coenzyme Q_{10} in patients with diabetes mellitus and insulin-resistant hypertension, respectively [14,15]. Scientific information has also demonstrated that antioxidant vitamins C and E are implicated in the improvement of endothelial function in humans and in animal models of diseases including hypertension [7,16,17]. Vitamin C alone or in combination with vitamin E has been shown to enhance nitric oxide (NO) generation and reduce blood pressure in hypertensive animals. Moreover, diet supplemented with vitamin E has been shown to enhance total antioxidant status and reduce blood pressure in SHR [7]. These two antioxidant vitamins are known to stimulate NO generation in endothelial cells and have ROS-scavenging effects [18,19], but the underlying mechanisms of action are unknown. Vitamin E reduces the susceptibility of low density lipoproteins (LDL) oxidation and decreases *in vivo* markers of lipid oxidation. Vitamin E, like β-carotene, may also act at the cellular level by

* Corresponding author. Tel.: +34 923294530; fax: +34 923294515.
E-mail address: masevilla@usal.es (M.-Á. Sevilla).

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inhibiting membrane lipid peroxidation and the production of ROS [20].

On other hand, there are a number of reports indicating that astaxanthin, an oxicarotenoid (3,3′-dihydroxy-β,β-carotene-4,4′-dione), pigment responsible of the red coloration in wild salmonids, crustaceans, as well as some kelps and yeast [21], is a more powerful antioxidant than other carotenoids [22]. In various studies it has been demonstrated that astaxanthin shows a wide variety of biological activities from anti-inflammatory [23], to antitumoral [24,25]. Also an astaxanthin derivative was reported to have cardioprotective effect in a rat infarct model [26,27]. Recently, works in SHR suggest that astaxanthin may act by modulating the blood fluidity in hypertension and the antihypertensive effects of astaxanthin may be exerted through mechanisms including normalization of the sensitivity of the adrenoceptor sympathetic pathway [28,29]. Moreover it is reported that structural alterations in SHR vasculature (coronary artery and aorta) were ameliorated by astaxanthin [30]. Nevertheless, it is not known yet how astaxanthin improves the vascular function in different vessels, its capacity to prevent cardiac hypertrophy, remodeling of arteries in target organs and its capacity to reduce $^{\bullet}O_2^-$ production.

Therefore, the aim of this study was to investigate the effect of chronic administration of astaxanthin on the development of hypertension, cardiac hypertrophy, vascular remodeling, vascular $^{\bullet}O_2^-$ production and especially vascular reactivity, since this has not been reported elsewhere.

2. Materials and methods

All the experimental procedures were performed in accordance with the standards established by conventional guidelines for care and use of laboratory animals.

Male SHR supplied by Elevage Janvier (France) were used in this study. Housing conditions were thermostatically maintained at 24 °C and 60% humidity, and a 12 h light/dark cycle. The animals were housed for at least 1 week before the experiments, fed with a standard diet for adult maintenance (AIN-93-M) [31] and water *ad libitum*.

At 12 weeks of age, SHR were randomized based on baseline blood pressure values into 3 groups ($n = 10$ per group) to receive during 2 months: standard diet (SHR-Control), astaxanthin 75 mg/kg body weight per day (SHR-Axt 75) and 200 mg/kg body weight per day (SHR-Axt 200). Body weight and systolic blood pressure (SBP) of all the rats were recorded weekly or fortnightly and the intake of food and water every second day. The astaxanthin was added weekly to the food which was prepared by ourselves, according to the weight and food intake of animals. Preliminary studies were used for the selection of doses.

The SBP was measured in awake rats with an automated multichannel system, using the tail-cuff method with a photoelectric sensor (Niprem 546, Cibertec SA, Spain) as previously described [32]. SBP was measured before starting the study and every week during the treatment. Changes in SBP were calculated with respect to initial value in each animal.

At the end of the treatment, the rats were anaesthetized with sodium pentobarbital (60 mg/kg body weight, i.p). The carotid artery was cannulated and heparinized physiological solution was perfused. An incision in the femoral artery permitted the drainage of blood and perfusion liquid.

Following, the thoracic aorta artery and left kidney were extracted to perform the functional tests, also the heart and the right kidney were extracted and placed in Krebs solution of the following composition (in mM): NaCl, 118; KCl, 4.7; $CaCl_2$, 2.5; KH_2PO_4, 1.2; $MgSO_4$, 1.2; $NaHCO_3$, 25; glucose, 11. The pH of the solution after saturation with carbogen (95% O_2, 5% CO_2 mixture) was adjusted to 7.4.

2.1. Morphometric studies

As was indicated above, after sacrificing the animals, the heart and right kidney were extracted. The heart was immediately placed in the Krebs solution at 37 °C, aired with carbogen. The fatty tissue was carefully eliminated and the atria were separated, the right ventricle was sectioned and the left ventricle was with the intraventricular septum intact. They were carefully dried, eliminating excess liquid and were weighed separately. The relation between the weight of the left ventricle and the body weight was used as left ventricle hypertrophy index (LVH).

Once the aorta was extracted, was carefully cleaned of fat and connective tissue and cut into 3 mm rings. One ring was used for morphometric studies and the rest for functional assays.

Samples of right kidney and thoracic aorta were fixed, dehydrated and embedded in paraffin. Cross-sections of thickness 4 μm were stained with hematoxylin–eosin for the light microscopy study. Sections of the aorta and intrarenal arteries were examined and photographed using the optic microscope (Olympus BX50) with an attached high resolution camera (Olympus DP50). Images were analysed (Scion Image Software, Scion Corporation web site http://scioncorp.com/), and internal and external perimeters of the medial layer measured and converted into internal and external radii (R_i and R_e, respectively), according to the formula: perimeter = $2\pi R$, where $2R_i$ was the internal diameter or lumen (L), and $R_e - R_i$ the medial thickness (W_m). Medial cross-sectional area (CSA_m) was assessed as: $CSA_m = \pi(R_e^2 - R_i^2)$.

Intrarenal arteries with an external diameter between 20 and 60 μm were used as resistance arteries, and the aorta as conductance artery.

2.2. Functionality studies: aorta

Rings for reactivity assays were placed between stainless-steel hooks and set up in organ chambers filled with 5 mL of Krebs solution, gassed with carbogen and kept at 37 °C. One of the hooks was fixed to the bath and the other connected to an isometric force transducer (UF1; Harvard Apparatus Inc., USA). The force was recorded on a PC computer using software Chart version 3.4 and a PowerLab/800 data acquisition system (AD Instruments, U.K.). All the rings were allowed to equilibrate for 1 h at a resting tension of 2 g. The Krebs solution was periodically changed and tension was reset during this period. After contraction with phenylephrine (PE, 10^{-6} M), and at the steady-state maximal contraction, cumulative concentration–response curves were obtained for ACh (10^{-8} to 10^{-4} M) and sodium nitroprusside (SNP, 10^{-9} to 3×10^{-5} M). ACh curves were also obtained after incubation with indomethacin (5×10^{-5} M; 30 min).

In order to evaluate the production of basal NO, the contraction induced by PE (10^{-6} M) was assessed before and 30 min after aortic incubation with N_ω-nitro-L-arginine methyl ester hydrochloride (L-NAME, 10^{-4} M), an eNOS inhibitor.

In all the experiments, the relaxant responses to ACh and SNP were expressed as percentages of PE contraction.

2.3. Functionality studies: renal vascular bed

The left kidney was cannulated through the renal artery with a polyethylene catheter and carefully isolated and removed. After that it was connected simultaneously, through a three way valve, with a pressure transducer and to the perfusion system. The perfusion was carried out with the Krebs solution, gassed with carbogen at a temperature of 37 °C and a constant flow of 3 mL/min using a perfusion pump (Masterflex L/S, Cole-Palmer, USA). Pressure changes were measured with a pression transducer and recorded on a computerized system PowerLab/800 for Windows.

After 15 min of stabilization, the tissue functionality was tested by perfusing 40 mM KCl until a stable contraction plateau has been reached. At this moment the drug was withdrawn from the perfusion fluid in order to recover the pressure of basal perfusion. The concentration–response curve to ACh (10^{-9} to 10^{-4} M) was carried out after the tissue contraction with PE (10^{-6} M). Once the stable contraction plateau was reached, solutions with 10^{-6} M PE plus increasing concentrations of ACh were perfused, waiting for a stable response before incorporating the next concentration.

Endothelium-independent relaxation to SNP (10^{-9} to 10^{-4} M) was assessed in PE (10^{-6} M) precontracted kidney.

In order to evaluate the formation of basal NO, the contraction induced by PE (10^{-6} M) was assessed as perfusion pressure changes, with and without L-NAME 10^{-4} M.

In all the experiments the relaxant responses to ACh and SNP were expressed as percentages of PE contraction.

2.4. Detection of vascular $^{\bullet}O_2^-$ production

The $^{\bullet}O_2^-$ production was assessed by lucigenin-enhanced chemiluminescence assay [5]. Briefly, rings of thoracic aorta were incubated in HEPES-buffer (in mM: NaCl, 119; HEPES, 20; $MgSO_4$, 1; KCl, 4.6; KH_2PO_4, 0.4; Na_2HPO_4, 0.15; $NaHCO_3$, 5; $CaCl_2$, 1.2; glucose, 5.5; pH, 7.4) gassed with carbogen and maintained at 37 °C for 30 min. Then samples were transferred into tubes containing 1 mL of HEPES-buffer with lucigenin (5 μM). Lucigenin chemiluminescence was then recorded every 30 s for 5 min in a luminometer (Lumat LB-9507, Berthold Technologies, Germany). Basal and NADPH-stimulated (100 μM) production were measured and expressed as relative luminescence units (RLU)/min/mg dry tissue.

3. Statistical analysis

Data are expressed as mean ± SEM. Concentration–response curves were analyzed using the GraphPad Prism 4.0 software (GraphPad, USA) and fitted to a logistic equation and from these plots the maximal relaxant effect (E_{max}) and the negative logarithm of the concentration producing half maximal relaxation (pD_2) were calculated. Statistical calculations for significant differences were performed using Student's *t* test in order to compare two groups or one-way ANOVA followed by a Newman–Keuls test. $P < 0.05$ was considered significant.

4. Drugs

The drugs used were phenylephrine hydrochloride, acetylcholine chloride, sodium nitroprusside, N,N-dimethyl-9,9-biacridinium dinitrate (lucigenin), NADPH tetrasodium salt, indomethacin, haematoxylin and eosin, all purchased from Sigma Chemical Co., USA. Astaxanthin was kindly supplied by BASF (BASF Laboratories, Barcelona, Spain).

Stock solution of drugs were made up in ultra-pure water and stored at −20 °C and appropriate dilutions were made daily.

5. Results

5.1. Systolic blood pressure

At the beginning of the study, the SBP was similar for the three groups (SHR-Control, 167 ± 3 mmHg; SHR-Axt 200, 169 ± 3 mmHg; and SHR-Axt 75, 163 ± 2 mmHg, $P = 0.23$). All the values were in agreement to the hypertensive state of the animals. Throughout the experiment the increase in SBP only was observed in SHR-Control group, in contrast, the two groups treated with astaxanthin showed a significant dose-dependent reduction in SBP values (Fig. 1).

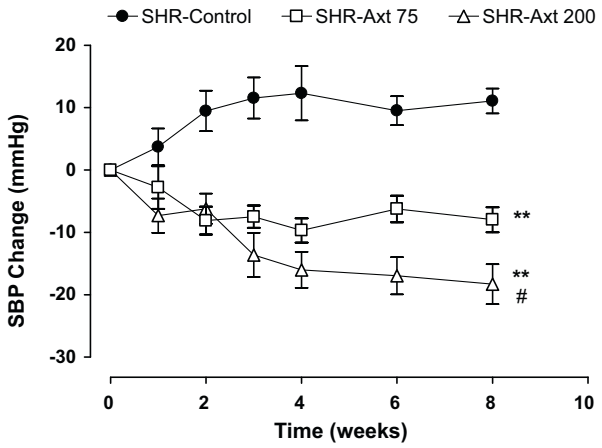

Fig. 1. Time course systolic blood pressure (SBP) changes in the spontaneously hypertensive rat control group (SHR-Control, $n = 10$), SHR astaxanthin treated with 75 mg/kg per day (SHR-Axt 75, $n = 10$) and SHR astaxanthin treated with 200 mg/kg per day (SHR-Axt 200, $n = 10$). Values are expressed as means ± SEM. $^{**}P < 0.01$ vs. SHR-Control group; $^{\#}P < 0.05$ vs. SHR-Axt 75 group.

Food intake did not differ significantly among groups during the study: 8.1 ± 0.2; 8.3 ± 0.3 and 7.9 ± 0.3 g/100 g body weight per day for SHR-Control, SHR-Axt 75 and SHR-Axt 200 respectively. There were also no differences in body weight (data not shown).

5.2. Morphometric studies

The left ventricular weight and the LVH in SHR-Control were significantly greater than in SHR-Axt 75 and SHR-Axt 200. The treatment with astaxanthin leads to an improvement in cardiac hypertrophy in both groups (Fig. 2).

SHR-Axt 75 and SHR-Axt 200 reduced significantly the media thickness and media cross-sectional area on both conductance and resistance arteries (Table 1). Only lower dose of astaxanthin was able to increase the lumen size (Table 1). This lead to smaller values in the medial thickness/lumen ratio (W_m/L) in SHR-Axt 75 compared with the SHR-Control and SHR-Axt 200 (Fig. 3(a and b)).

5.3. Functionality studies: aorta

ACh-induced relaxations were not modified significantly in intact aortic rings from SHR-Axt 200 ($E_{max} = 45.6 \pm 0.9\%$;

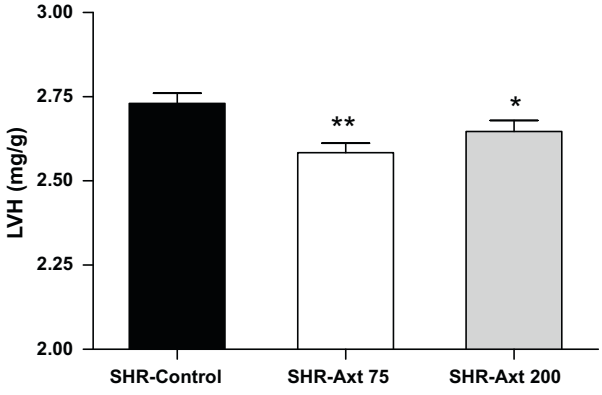

Fig. 2. Relation between the weight of the left ventricle and the body weight, used as left ventricular hypertrophy index (LVH), in spontaneously hypertensive rat control group (SHR-Control, $n = 10$), SHR astaxanthin treated with 75 mg/kg per day (SHR-Axt 75, $n = 10$) and SHR astaxanthin treated with 200 mg/kg per day (SHR-Axt 200, $n = 10$). Values are expressed as means ± SEM. $^{*}P < 0.05$ and $^{**}P < 0.01$ vs. SHR-Control group.

Table 1
Wall geometry of thoracic aortic and small intrarenal resistance arteries.

Aorta	L (m)	W_m (m)	CSA_m (mm^2)
SHR-Control	1514 ± 18	130.0 ± 3.4	0.659 ± 0.014
SHR-Axt 75	1636 ± 31**	112.0 ± 3.9**	0.597 ± 0.018**
SHR-Axt 200	1519 ± 21	115.3 ± 3.0*	0.589 ± 0.014*
Intrarenal arteries	L (μm)	W_m (μm)	CSA_m (μm^2)
SHR-Control	17.3 ± 0.8	10.3 ± 0.4	847.6 ± 55.9
SHR-Axt 75	21.6 ± 1.0**	8.0 ± 0.3**	707.3 ± 50.0*
SHR-Axt 200	18.0 ± 0.9	8.6 ± 0.4**	710.6 ± 58.2*

L, lumen; W_m, medial thickness; CSA_m, medial cross-sectional area. Spontaneously hypertensive rat control group (SHR-Control), SHR astaxanthin treated with 75 mg/kg body weight per day (SHR-Axt 75) and SHR astaxanthin treated with 200 mg/kg body weight per day (SHR-Axt 200). Values are means ± SEM (n = 5 in each group).

* $P < 0.05$ vs. SHR-Control group.

** $P < 0.01$ vs. SHR-Control group.

$pD_2 = 7.4 \pm 0.2$) and SHR-Axt 75 ($E_{max} = 49.0 \pm 2.1\%$; $pD_2 = 7.2 \pm 0.1$) compared with SHR-Control ($E_{max} = 45.9 \pm 1.6\%$; $pD_2 = 7.3 \pm 0.2$) (Fig. 4(a)). Exposure of aorta rings to indomethacin increased ACh-induced vasorelaxation in all the experimental groups SHR-Axt 200 ($E_{max} = 77.8 \pm 3.1\%$; $pD_2 = 7.3 \pm 0.5$), SHR-Axt 75 ($E_{max} = 83.4 \pm 2.7\%$; $pD_2 = 7.0 \pm 0.2$), and SHR-Control ($E_{max} = 82.6 \pm 3.5\%$; $pD_2 = 7.6 \pm 0.7$); but these increases did not produce differences among the astaxanthin-treated groups and control (Fig. 4(b and c)).

Also, no differences were observed in the endothelium-independent relaxant responses to SNP in aortic rings from the

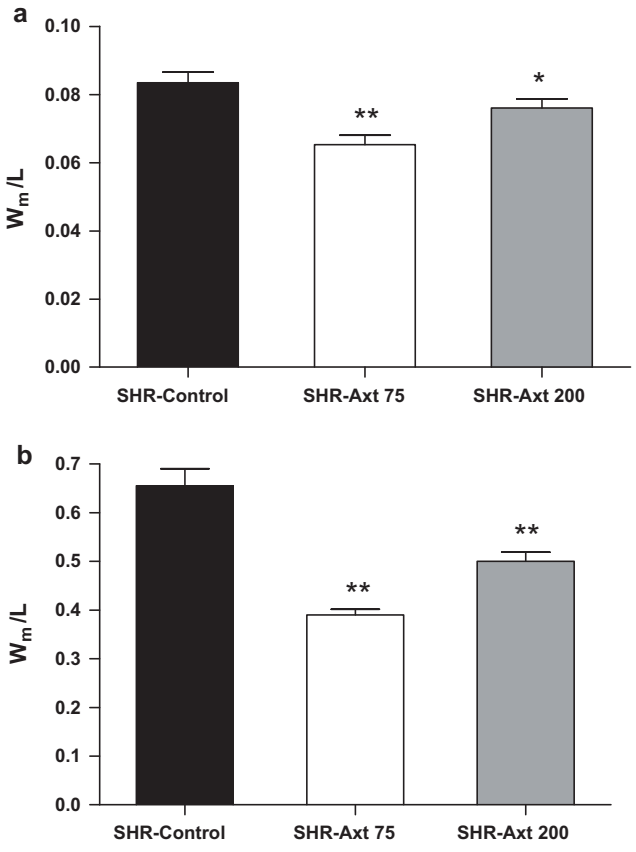

Fig. 3. Medial thickness/lumen ratio (W_m/L) from spontaneously hypertensive rat control group (SHR-Control, n = 5), SHR astaxanthin treated with 75 mg/kg per day (SHR-Axt 75, n = 5) and SHR astaxanthin treated with 200 mg/kg per day (SHR-Axt 200, n = 5). (a) Aorta, (b) intrarenal arteries. Values are expressed as means ± SEM. *$P < 0.05$ and **$P < 0.01$ vs. SHR-Control group.

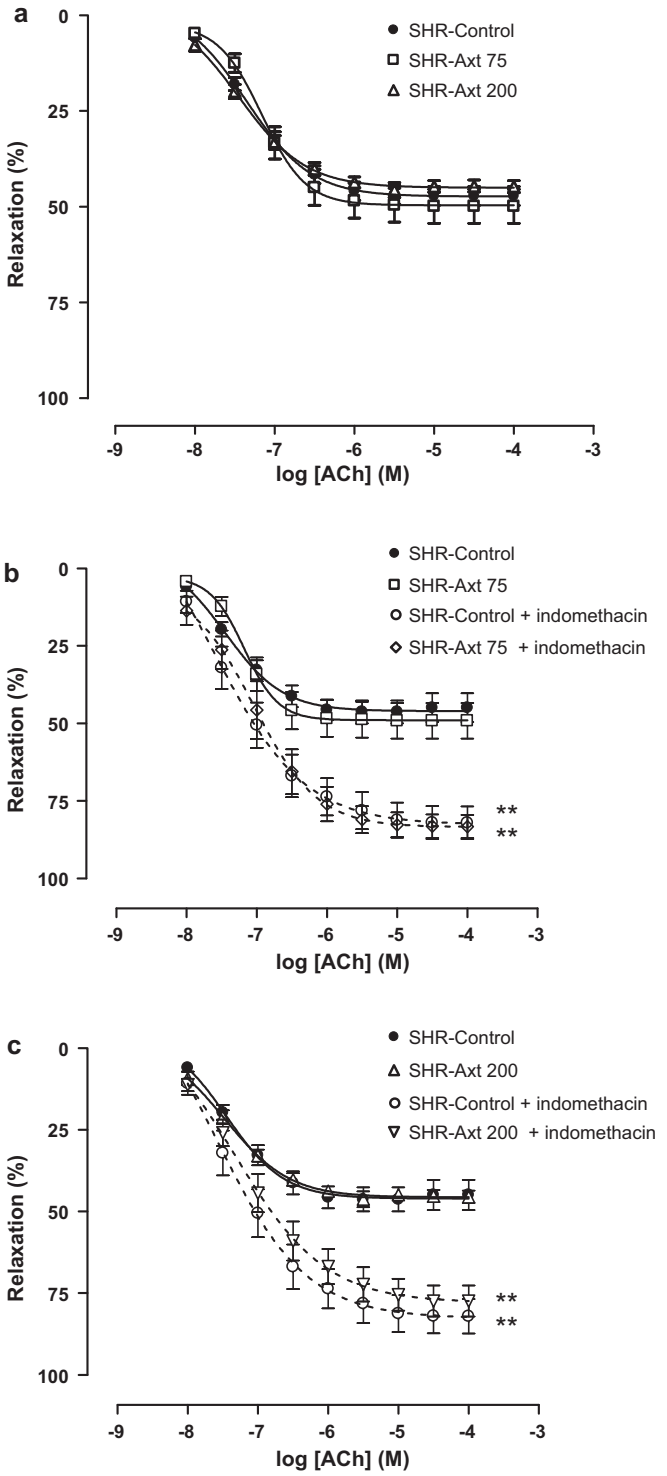

Fig. 4. (a) Relaxations induced by acetylcholine (ACh), in aorta from spontaneously hypertensive rat control group (SHR-Control, n = 9), SHR astaxanthin treated with 75 mg/kg per day (SHR-Axt 75, n = 10) and SHR astaxanthin treated with 200 mg/kg per day (SHR-Axt 200, n = 10). (b) Relaxations induced by ACh in aorta with and without indomethacin incubation from SHR-Axt 75. (c) Relaxations induced by ACh in aortas with and without indomethacin incubation from SHR-Axt 200. Values are expressed as means ± SEM. **$P < 0.01$ vs. the corresponding group without indomethacin incubation.

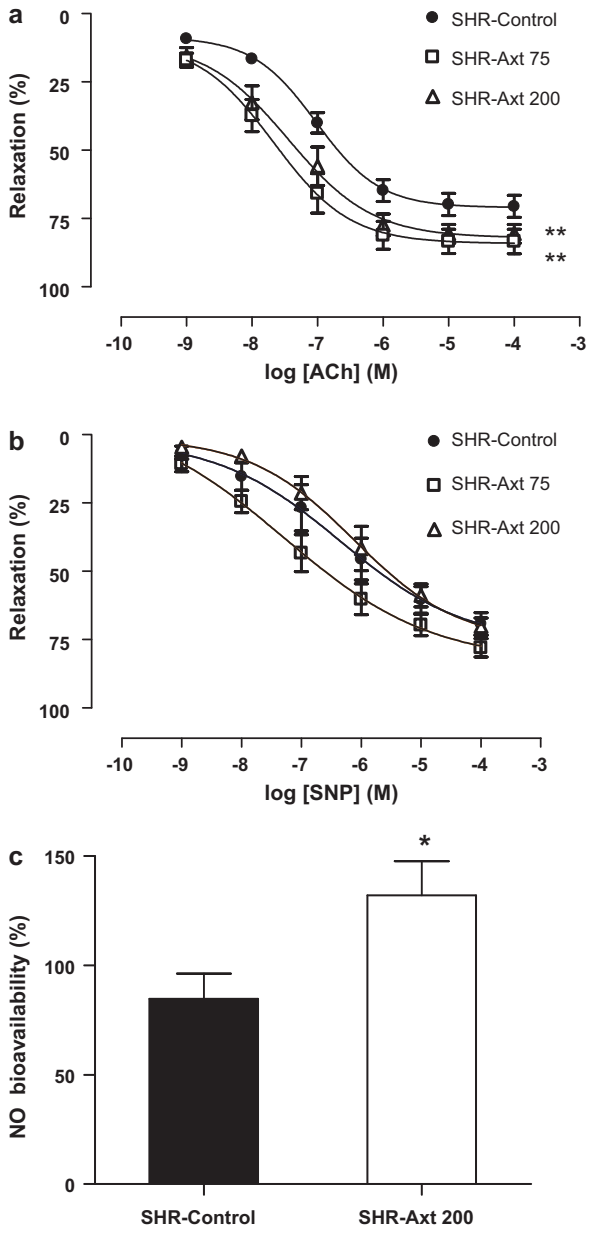

Fig. 5. (a) Vascular relaxation to acetylcholine (ACh) in intrarenal arteries from spontaneously hypertensive rat control group (SHR-Control, $n = 9$), SHR astaxanthin treated with 75 mg/kg per day (SHR-Axt 75, $n = 10$) and SHR astaxanthin treated with 200 mg/kg per day (SHR-Axt 200, $n = 9$). (b) Vascular relaxation to sodium nitroprusside (SNP) in intrarenal arteries from all groups. (c) Basal NO production (NO bioavailability) in SHR-Control and SHR-Axt 200. Values are expressed as means ± SEM. $^{*}P < 0.05$ and $^{**}P < 0.01$ vs. SHR-Control group.

three groups: SHR-Axt 200 ($E_{max} = 77.6 \pm 3.9\%$; $pD_2 = 7.5 \pm 0.2$), SHR-Axt 75 ($E_{max} = 75.5 \pm 3.4\%$; $pD_2 = 7.6 \pm 0.2$), and SHR-Control ($E_{max} = 79.7 \pm 4.7\%$; $pD_2 = 7.7 \pm 0.3$). These findings suggest that astaxanthin did not modify endothelial dependent and independent function in aorta from SHR.

Basal NO bioavailability was not significantly different among all experimental groups (data not shown).

5.4. Functionality studies: renal vascular bed

ACh relaxations were significantly increased in the renal vascular bed from SHR-Axt 200 ($E_{max} = 82.1 \pm 3.8\%$; $pD_2 = 7.4 \pm 0.3$) and SHR-Axt 75 ($E_{max} = 84.2 \pm 3.8\%$; $pD_2 = 7.7 \pm 0.3$) compared with SHR-Control ($E_{max} = 71.1 \pm 2.9\%$; $pD_2 = 7.0 \pm 0.1$) (Fig. 5(a)).

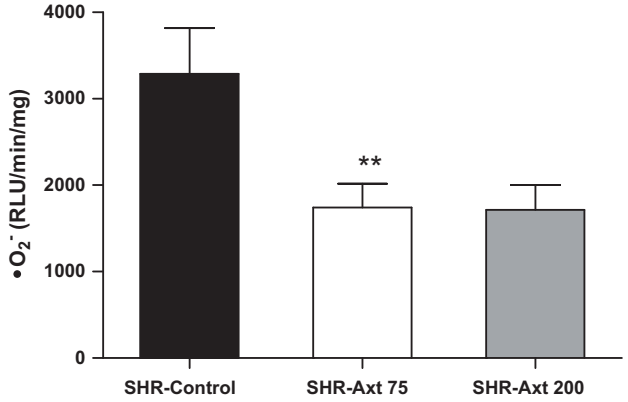

Fig. 6. Vascular superoxide anion ($^{\bullet}O_2^-$) level expressed as relative luminescence units (RLU)/min/mg dry tissue stimulated by NAD(P)H addition. Aortic rings from spontaneously hypertensive rat control group (SHR-Control, $n = 10$), SHR astaxanthin treated with 75 mg/kg per day (SHR-Axt 75, $n = 8$) and SHR astaxanthin treated with 200 mg/kg per day (SHR-Axt 200, $n = 10$). Values are expressed as means ± SEM. $^{*}P < 0.05$ vs. SHR-Control group.

In contrast, the maximun endothelium-independent relaxant responses to SNP did not show significant differences among the three groups SHR-Axt 200 ($E_{max} = 75.9 \pm 10.6\%$; $pD_2 = 6.1 \pm 0.3$), SHR-Axt 75 ($E_{max} = 83.4 \pm 12.2\%$; $pD_2 = 7.3 \pm 0.7$), and SHR-Control ($E_{max} = 76.4 \pm 15.8\%$; $pD_2 = 6.3 \pm 0.5$), although the lower dose of astaxanthin showed a higher pD_2 value (Fig. 5(b)).

The basal NO bioavailability was significantly higher in SHR-Axt 200 group than SHR-Control $P < 0.05$ (Fig. 5(c)).

5.5. Vascular $^{\bullet}O_2^-$ production

No significant differences were found in basal vascular production of $^{\bullet}O_2^-$ among the experimental groups, as measured by chemiluminescence lucigenin. Aortic stimulation with NADPH 100 μmol/L enhanced the basal production of $^{\bullet}O_2^-$ from 6 to 10 times in all the experimental groups, but the increase was significantly higher in the SHR-Control than SHR-Axt 200 and SHR-Axt 75 (Fig. 6). These results indicate that astaxanthin was capable of reducing the $^{\bullet}O_2^-$ levels in both the doses.

6. Discussion

Experimental evidences indicate that ROS play an important pathophysiological role in hypertension and treatment with antioxidants improves vascular and renal function, regresses vascular remodeling and reduces blood pressure [33]. Astaxanthin is a powerful antioxidant, but as far as we know only three studies have been conducted about its role in hypertension [28–30].

Astaxanthin supplemented diet reduced the SBP, at both doses tested in SHR 12-week-old which have already established hypertension. SHR is a well-known animal model of hypertension in which the oxidative stress is increased. Hussein et al. also reported, in hypertensive rats, decreases in blood pressure of about 10–25 mmHg with 5 and 50 mg/kg per day of astaxanthin administered chronically, but did not have an effect in normotensive animals [28,29]. There are some studies using vitamins C and E as antihypertensive agents in SHR. In all the cases, independently of dose and time, the best effect was a 30 mmHg reduction in SBP [7,18,19,34,35]. Also, with others dietary antioxidants such as flavonoids, the mean reduction report has been about 20 mmHg in SHR [36]. These antihypertensive effects have been associated directly to antioxidant properties or a reduction in $^{\bullet}O_2^-$ production.In our study, we show for the first time that astaxanthin reduces ventricular hypertrophy in hypertensive animals. This

effect is, at least partly, independent of blood pressure reduction as there were no differences in LVH rates between the doses of 75 and 200 mg/kg body weight per day. We believe that the reduction in superoxide radical production, which was similar with both doses as shown by the results of chemioluminescence, is primarily responsible. Our results are in keeping with other works which report that vitamin C and other antioxidants such as quercetin and wine polyphenols also induce a reduction of the LVH values in SHR [35–37].

Vascular remodeling significantly contributes to pathophysiology of vascular diseases, including hypertension [38]. It is associated with alterations of the luminal diameter and changes in tunica media mass. Interestingly astaxanthin improved vascular remodeling in both conductance and resistance vessels. In astaxanthin-treated groups the media thickness was significantly reduced in comparison with the SHR-Control group, and CSA_m as well as W_m/L ratio were also decreased. As we mentioned above antioxidant astaxanthin properties related with a lower $^{\bullet}O_2^-$ production could be implicated in this beneficial effect. These findings are in concordance with data reported by Hussein et al. [30]. Their astaxanthin-treated SHR group exhibited thinner wall thickness, wider lumens, and deceased W_m/L ratio compared to the controls in coronary arteries. Similar effects have been reported using vitamin C in SHR renal vascular bed [35], and with vitamins C and E in SHR mesenteric arteries [7]. These facts reinforce the relation between antioxidant dietary intake and blood pressure reduction, and amelioration of remodeling and endothelial dysfunction associated to hypertension [39–41].

In the present study, astaxanthin did not improve endothelium-dependant relaxation in thoracic aortas of SHR. Some authors have also reported this fact [28]. These data do not agree with other studies, where it has been described that the antioxidant vitamins used improve endothelium-dependent relaxation [16,42] by increases in eNOS activity and NO generation in SHR arteries [18,19]. As expected, the indomethacin augmented the endothelium-dependent relaxation by inhibition of the COX pathway, but this increased relaxation did not produce differences among the astaxanthin-treated groups and SHR-Control [43]. Astaxanthin was unable to modify the participation of prostanoids in the endothelium-dependent relaxation.

On the other hand, SNP produced the same degree of vasorelaxation in the thoracic aortas of all experimental groups, these results are consistent with the mentioned previous studies [42,43].

Endothelium-independent vasodilation induced by SNP was also unaffected by astaxanthin in perfussed kidney. On the contrary, astaxanthin improved endothelial-dependent relaxation renal vascular bed. In the present study, astaxanthin in both treated groups (SHR-Axt 75 and SHR-Axt 200) produced a significant increase in E_{max} values about 18% to 15% respectively. It is remarkable, as far we know that there are no reports about the effect of astaxanthin on endothelium-dependent relaxation in resistance vessels. Putative mechanisms whereby antioxidants improve endothelial function may be due to direct ROS scavenging ability [7,44,45], their capability to upregulate eNOS activity by increasing intracellular BH_4, which would increase NO [18,19], their ability to regulate NAD(P)H oxidase and/or antioxidant enzymes [7] Besides, these findings are consistent with two facts from this study. On the one hand treatment with astaxanthin was able to reduce the NADPH enhanced $^{\bullet}O_2^-$ production, although had no effect on basal production of $^{\bullet}O_2^-$, as evidenced by chemiluminescence assays and the fact that the bioavailability of NO are not modified in aorta ring. On the other hand, astaxanthin improved endothelial function in resistance arteries, what could be due to increased NO bioavailability assessed by the PE-induced contraction before and 30 min after incubation with L-NAME.Taken together our results we think that relaxation improvement in vascular renal bed produced in treated groups was due to changes in endothelium-derived NO activity, rather than downstream transduction on vascular smooth muscle cells. The improved relaxant response in this vascular bed could be associated to an increased bioavailability of NO results of a reduction in $^{\bullet}O_2^-$ production.

It is noteworthy that we have only observed a relationship dose–effect with astaxanthin in reducing blood pressure. The beneficial effects of both dose in the others evaluated cardiovascular parameters were similar or even higher at the lower dose assayed. Despite the substantial evidence that oxidative stress contributes to hypertension, there is not a clear understanding of exactly how this happens. A relatively new direction is the concept that inflammatory response involving oxidative stress contributes to hypertension [46]. Further investigations with astaxanthin in this way might be interesting to clarify its antihypertensive mechanism and possible therapeutic benefit.

7. Conclusion

The major findings to be drawn from this study are that astaxanthin exerts a blood pressure lowering effect that is associated with improved endothelium-dependent vasodilatation in resistance vessels, improvements in cardiovascular remodeling and decreases $^{\bullet}O_2^-$ production stimulated by NAD(P)H oxidase. All these effects of astaxanthin could be beneficial for the management of arterial hypertension.

Conflict of interest

All the authors declare that they have no conflict of interest with any person or in any firm related to this work.

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