

Cardiovascular Response to Chronic Administration of C-type Natriuretic Peptide in Spontaneously Hypertensive Rats

Respuesta cardiovascular a la administración crónica de péptido natriurético tipo C en ratas espontáneamente hipertensas

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ABSTRACT

Background: C-type natriuretic peptide (CNP) plays an important role in the regulation of cardiovascular function and morphology. We have previously demonstrated that CNP increases nitric oxide (NO) system activity in vivo in spontaneously hypertensive rats (SHR).

Objective: The goal of this study is to evaluate the effect of chronic CNP administration on systolic blood pressure (SBP), cardiovascular function and the NO system in spontaneously hypertensive and normotensive rats.

Methods: Twelve-week-old normotensive male Wistar rats and SHR were used. They received chronic infusion of saline or CNP (0.75 µg/h/rat) for 14 days via subcutaneously implanted osmotic pumps. Systolic blood pressure was measured and an electrocardiogram and echocardiogram were performed. The left ventricle and the thoracic aorta were resected; nitric oxide synthase (NOS) activity was determined using L-[U14C]-arginine and vascular reactivity was assessed.

Results: Chronic administration of CNP decreased SBP in SHR. Cardiac output was lower in SHR and increased with CNP; however, CNP had no effect in normotensive rats. Spontaneously hypertensive rats had unbalanced aortic vasodilation and vasoconstriction responses, and CNP improved the vascular function. Nitric oxide synthase activity was greater in SHR and increased with the 14-day CNP infusion, but this increase was lower than in normotensive rats.

Conclusion: C-type natriuretic peptide induces cardiovascular and NO system changes which may be beneficial in this model of hypertension.

Key words: C-type Natriuretic Peptide - Systolic Arterial Pressure - Heart - Aortic Artery - Nitric Oxide Synthase

RESUMEN

Introducción: El péptido natriurético tipo C (CNP) ha cobrado relevancia por sus efectos sobre la regulación de la función y la morfología del corazón y los vasos sanguíneos. Previamente demostramos in vitro que el CNP incrementa la actividad del sistema del óxido nítrico (NO) en ratas espontáneamente hipertensas (SHR).

Objetivo: Estudiar el efecto del tratamiento crónico con CNP sobre la presión arterial sistólica (PAS), la función cardíaca y vascular y el sistema del NO en ratas espontáneamente hipertensas y normotensas.

Material y métodos: Se emplearon ratas Wistar macho de 12 semanas de edad normotensas y espontáneamente hipertensas. Los animales recibieron infusión crónica de solución salina o CNP (0,75 µg/hora/rata) durante 14 días mediante la implantación de bombas osmóticas subcutáneas. Se midió la PAS y se realizaron un electrocardiograma y un ecocardiograma. Se extrajeron el ventrículo izquierdo y la arteria aorta torácica y se determinó la actividad, con L-[U14C]-arginina, de la óxido nítrico sintasa (NOS) y se realizaron estudios de reactividad vascular.

Resultados: La administración crónica de CNP disminuyó la PAS en las SHR. Se observó menor volumen minuto en las SHR y el CNP incrementó dicho volumen, en tanto que no indujo cambios en las ratas normotensas. En las SHR se observó un desequilibrio en las respuestas vasodilatadora y vasoconstrictora en la arteria aorta y el tratamiento con CNP mejoró la función vascular respecto de las ratas normotensas. En ambos tejidos, la actividad de la NOS fue mayor en las SHR y se incrementó con la infusión durante 14 días de CNP. Sin embargo, dicho incremento fue menor en las SHR.

Conclusión: El CNP induce cambios a nivel cardiovascular y en el sistema del NO que podrían resultar beneficiosos en este modelo de hipertensión arterial.

Palabras clave: Péptido natriurético tipo C - Presión arterial - Corazón - Arteria aorta - Óxido nítrico sintasa

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Abbreviations

CNP	C-type natriuretic peptide	SBP	Systolic blood pressure
LVDD	Left ventricular diastolic dimension	SHR	Spontaneously hypertensive rats
HTN	Hypertension	SS	Saline solution
LVMI	Left ventricular mass index	EDV	End diastolic volume
NO	Nitric oxide	LV	Left ventricle
NOS	Nitric oxide synthase	CO	Cardiac output
NPR-C	Natriuretic peptide receptor-C	SV	Stroke volume
BP	Blood pressure		

INTRODUCTION

Hypertension (HTN) is the most important risk factor for cardiovascular diseases and still represents a challenge for public health worldwide. In the RENATA study (Registro Nacional de Hipertensión Arterial, National Registry of Hypertension trial, performed by the Argentine Council of Hypertension of the Argentine Society of Cardiology) 4006 subjects surveyed from all the regions of the country (CABA, Buenos Aires, Córdoba, Tucumán, Corrientes, Chaco, Mendoza and Neuquén) showed a prevalence of 33.5% HTN. (1) Over the last decades, treatment of HTN has achieved significant progress with the use of renin-angiotensin system inhibitors: angiotensin-converting enzyme inhibitors and angiotensin receptor blockers, diuretics, adrenergic antagonists and calcium channel blockers. Despite these advances, blood pressure (BP) control has been extremely difficult to achieve in 40% of hypertensive patients. The results of the RENATA trial showed that 52.8% of hypertensive patients treated could not have adequate BP control. For these reasons, the development of new therapeutic strategies is still a growing target, and all that allows elucidating the mechanisms which produce and maintain HTN and the associated conditions, not only contributes to keep BP control within normal values but also helps to reduce target organ injury, the chief culprit of quality of life deterioration and risk of cardiovascular events.

Spontaneously hypertensive rats (SHR) constitute an adequate animal model of genetic hypertension to study essential hypertension in Humans, as they reproduce many characteristics of this condition. As it happens with essential hypertension in humans, hypertension in SHR is associated with complications of several organs, as the heart and vessels. (2) Among other alterations, remodeling involves myocardial hypertrophy, reduced coronary vascular function, interstitial and perivascular fibrosis and imbalance in collagen production. (3) The vascular abnormalities include changes in the intima-media thickness and in the composition of the extracellular matrix. (4) In addition, SHR present endothelial dysfunction, increased oxidative stress and vasoconstrictor factors, and decreased nitric oxide (NO) bioavailability. (5, 6) Alvarez et al. demonstrated that oxidative stress could be both cause and consequence for the development of

hypertrophy in this model of genetic hypertension, (7) and it has been shown that reactive oxygen species, which are increased in hypertension, reduce NO bioavailability. (8)

Although atrial and B-type natriuretic peptides have been widely studied, particularly for their diagnostic properties, C-type natriuretic peptide (CNP) has become more relevant due to its effects on the regulation of the structure and function of the heart and blood vessels. (8) This peptide is widely distributed in the cardiovascular system, especially in the endothelial cells, fibroblasts and cardiomyocytes (9) and, thus, occupies a strategic place in the mechanisms involved in BP regulation and in the protection of the heart and vessels against HTN. Previous studies performed in our laboratory demonstrated that the acute administration of CNP reduces BP in normotensive rats and SHR, and this effect is associated with higher NO system activity in both groups of animals. (10, 11) We also demonstrated that CNP increases nitric oxide synthase (NOS) activity in the heart and vessels due to the interaction with the natriuretic peptide receptor-C (NPR-C) coupled to the Gi protein. (10, 11)

Thus, the goal of this study was to evaluate the effect of chronic administration of CNP on systolic blood pressure (SBP), cardiovascular function and the NO system in normotensive young adult rats and SHR.

METHODS

Animals

Twelve-week-old male Wistar and SHR rats, with body weight between 250 and 300 g, were used. Animals were housed in a humidity- and temperature-controlled, with and automatic 12-hour light/dark cycle, and were fed with standard rat chow (Nutrimentos Purina) and tap water ad libitum until the end of the experimental period.

Experimental design

Tail-cuff SBP was measured in conscious rats at 12 weeks of age. The animals were randomly divided into four experimental groups: Wistar-SS, Wistar-CNP, SHR-SS and SHR-CNP, receiving chronic infusion with saline solution (SS, NaCl 0.9%) or CNP (0.75 µg/h/rat) for 14 days via subcutaneously implanted osmotic pumps. (12)

At day 12, animals were sedated with ketamine (100 g/kg, i.p.) and midazolam (5 g/kg, i.p.) and the ventral thorax and upper abdomen were shaved using aseptic techniques. Echocardiography evaluation was performed in the left lateral de-

cubitus position. Two-dimensional and M-mode images were obtained using a SonoScape ultrasound machine (A6Vet, with a multi-frequency electronic micro-convex array transducer 9-4 MHz). Heart rate (beats/minute) was monitored with standard electrocardiogram (Temis TM 1210). Ventricular volumes were calculated using the following formulas: $EDV = [(LVDD)^3/1.000]$; $ESV = [(LVSD)^3/1.000]$; $SV = [EDV - ESV]$; $CO = [(EDV - ESV) \times HR]$. (13)

Systolic BP was recorded again at the end of the experimental phase using the tail-cuff method and the animals were killed by decapitation. Then, the descending thoracic aorta was removed to determine vascular reactivity, and the left ventricle (LV) was removed to determine left ventricular mass index (LVMI), expressed as LV weight (g)/tibia length (mm); NOS activity was measured in both tissues.

Vascular reactivity

The aorta was cut into rings (3-5 mm) which were suspended in Krebs solution, bubbled with 95% O₂/5% CO₂ at 37 ± 1°C and pH 7.4. Each ring was progressively stretched to an optimal resting tension of 1 g and allowed to equilibrate for 60 min. Then, isometric tension (g) was measured using a force transducer connected to a polygraph (TRI 201 PanLab Quad Bridge Amp; Power Lab Physiograph modules, ADInstruments), firstly using cumulative concentrations of phenylephrine (a selective agonist of α₁-adrenergic receptors, 10⁻¹⁰-10⁻⁵ M) and finally with cumulative concentrations of acetylcholine (ACh, 10⁻¹⁰-10⁻⁵ M). Data was processed with a data acquisition system (PowerLab 8/30 and Labchart 7.0, Australia).

Determination of nitric oxide synthase activity

Nitric oxide synthase activity was determined in cross-sections from the aorta and LV (2-3 mm) using [14C] L-arginine as substrate, according to a previously described technique. (10, 11, 14) NOS activity was expressed as pmol [14C] L-citrulline/g tissue/minute.

Statistical analysis

Results are expressed as mean ± standard error of the mean. Analysis of variance (ANOVA) was used, followed by Bonferroni post-hoc test for multiple comparisons. A p value < 0.05 was considered statistically significant.

Ethical considerations

Animals were treated according to the American Heart Association Guide for the Care and Use of Laboratory Animals and the ANMAT (National Drug Food and Medical Technology Administration), Argentina, regulation N° 6344/96. The experiments were approved by the Ethics Committee of the School of Pharmacy and Biochemistry, University of Buenos Aires.

RESULTS

Effect of C-type natriuretic peptide on systolic blood pressure and left ventricular function

Panel A, Figure 1, shows that, as expected, SBP values before treatment were higher in SHR compared with Wistar rats. Panel B shows the effect of chronic administration of CNP on SBP. The administration of CNP did not induce changes in blood pressure in normotensive rats but decreased SBP in SHR after 14 days of treatment (Figure 1).

Compared with normotensive rats, ventricular function parameters were modified in SHR, as shown in Table 1, with reductions in LV diastolic dimension (LVDD), end diastolic volume (EDV), stroke volume (SV) and cardiac output (CO). Chronic administration of CNP increased these parameters in SHR and did not produce changes in Wistar rats. However, there were no differences in the ejection fraction and shortening fraction (Table 1).

Table 2 shows that elevated BP levels characteristic of SHR were accompanied by left ventricular hypertrophy. Yet, chronic CNP administration did not induce significant changes in the LV/body weight ratio and LVMI in any group of animals (Wistar-SS=0.021±0.001; Wistar-CNP=0.021±0.001; SHR-SS=0.032±0.002*; SHR-CNP=0.029±0.001; n=6 rats/group; * p<0.01 vs. Wistar-SS).

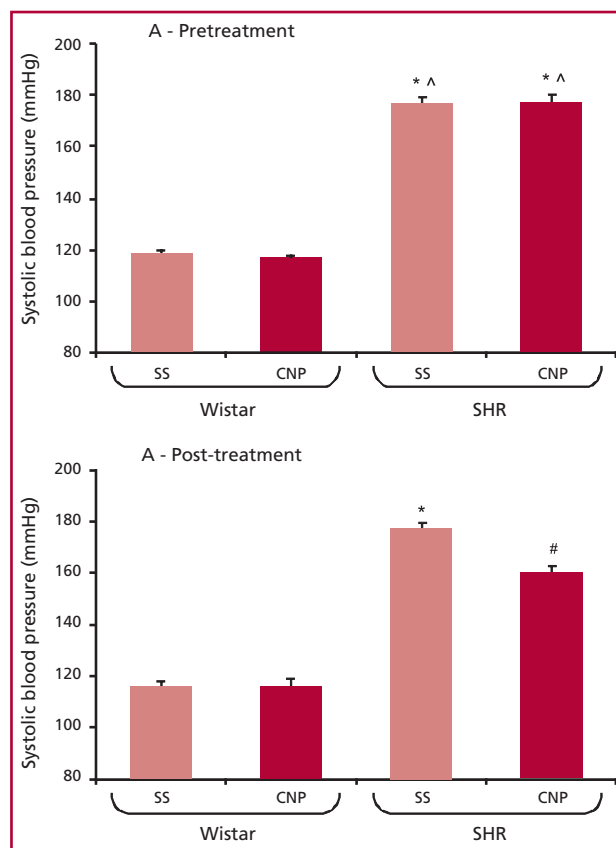


Fig. 1. Effect of chronic treatment with type-C natriuretic peptide (CNP) during 14 days on systolic blood pressure in normotensive and spontaneously hypertensive rats (SHR). Panel A: Baseline systolic blood pressure in Wistar rats and SHR. Panel B: Systolic blood pressure after 14 days of CNP or saline solution (SS) in Wistar rats and SHR. Statistical analysis: Analysis of variance (ANOVA), followed by Bonferroni post-hoc test for multiple comparisons. Results are expressed as mean ± standard error of the mean, n = 6 rats/group. * p < 0.01 vs. Wistar-SS; ^ p < 0.01 vs. Wistar-CNP; # p < 0.01 vs. SHR-SS

Effect of C-type natriuretic peptide on aortic reactivity

When aortic reactivity was evaluated with the administration of phenylephrine, we observed that although the maximal response was similar between both groups of animals, phenylephrine-induced vasoconstriction was greater in SHR than in Wistar rats. While the maximal vasoconstrictor response increased with chronic administration of CNP in normotensive rats, this was reduced in SHR (Figure 2, Panel A).

Maximal acetylcholine-induced vasodilation was lower in SHR compared with Wistar rats, and treatment with CNP for 14 days increased maximal acetylcholine-induced vasodilation and the vasodilator capacity in SHR (Figure 2, Panel B).

Effect of C-type natriuretic peptide on nitric oxide synthase activity in the aorta and left ventricle

Baseline NOS activity in the aorta and LV was higher in SHR compared with normotensive rats (Figure 3, Panels A and B). Chronic administration of CNP increased NOS activity in both tissues and in both groups of animals; however, this increase was lower both in the aorta and LV in SHR (Figure 3).

DISCUSSION

Our results show that treatment with CNP during 14 days decreased SBP in SHR but did not modify SBP

in normotensive rats. However, chronic administration of CNP increased NOS activity both in the LV and in the aorta in both groups of animals, and this increase was lower in SHR compared with Wistar rats. In addition, in previous studies performed in our laboratory, we demonstrated that acute infusion of CNP decreased mean BP in normotensive rats and SHR, and in both groups of animals this hypotensive effect was associated with higher NOS activity in the cardiovascular system. (10, 11) This difference observed in the hypotensive response to chronic and acute administration of CNP would indicate that although the NO system could be related to the hypotensive effect of the peptide in SHR, other mechanisms could compensate the hypotensive effect of CNP chronic treatment in normotensive rats.

Chronic administration of CNP increased vasoconstriction in normotensive rats treated with increasing concentrations of phenylephrine but this response was reduced in the aortic rings in SHR treated with CNP. The vasodilator effect of CNP in different vascular beds is widely known; several authors postulate that the activation of both type-B and type-C natriuretic receptors induces higher GMPc levels or the opening of potassium channels, inducing vascular smooth muscle relaxation. (15-18) Moreover, although treatment with CNP for 14 days

	Wistar		SHR	
	SS	CNP	SS	CNP
LVDD (mm)	6.0 ± 0.2	5.9 ± 0.3	5.4 ± 0.1*	6.1 ± 0.1#
LVSD (mm)	3.6 ± 0.1	3.3 ± 0.2	3.6 ± 0.2	4.0 ± 0.2
EDV (ml)	0.21 ± 0.03	0.22 ± 0.04	0.16 ± 0.02*	0.23 ± 0.03#
ESV (ml)	0.05 ± 0.01	0.04 ± 0.01	0.05 ± 0.01	0.06 ± 0.01
SV (ml)	0.17 ± 0.02	0.18 ± 0.02	0.11 ± 0.01*	0.17 ± 0.01#
HR (bpm)	429 ± 16	438 ± 10	435 ± 10	434 ± 15
CO (ml/min)	80.8 ± 8.1	92.4 ± 6.3	47.9 ± 2.0*	75.8 ± 3.1#
EF (%)	77 ± 2	82 ± 2	70 ± 6	71 ± 5
SF (%)	39 ± 2	44 ± 3	34 ± 5	36 ± 4

SHR: Spontaneously hypertensive rats. SS: Saline solution. CNP: C-type natriuretic peptide. LVDD: Left ventricular diastolic dimension. LVSD: Left ventricular systolic dimension. EDV: End diastolic volume. ESV: End systolic volume. SV: Stroke volume. HR: Heart rate. CO: Cardiac output. EF: Ejection fraction. SF: Shortening fraction. Statistical analysis: Analysis of variance (ANOVA), followed by Bonferroni post-hoc test for multiple comparisons. Results are expressed as mean±standard error of the mean, n = 6 rats/group. * p < 0.05 vs. Wistar-SS; # p < 0.05 vs. SHR-SS

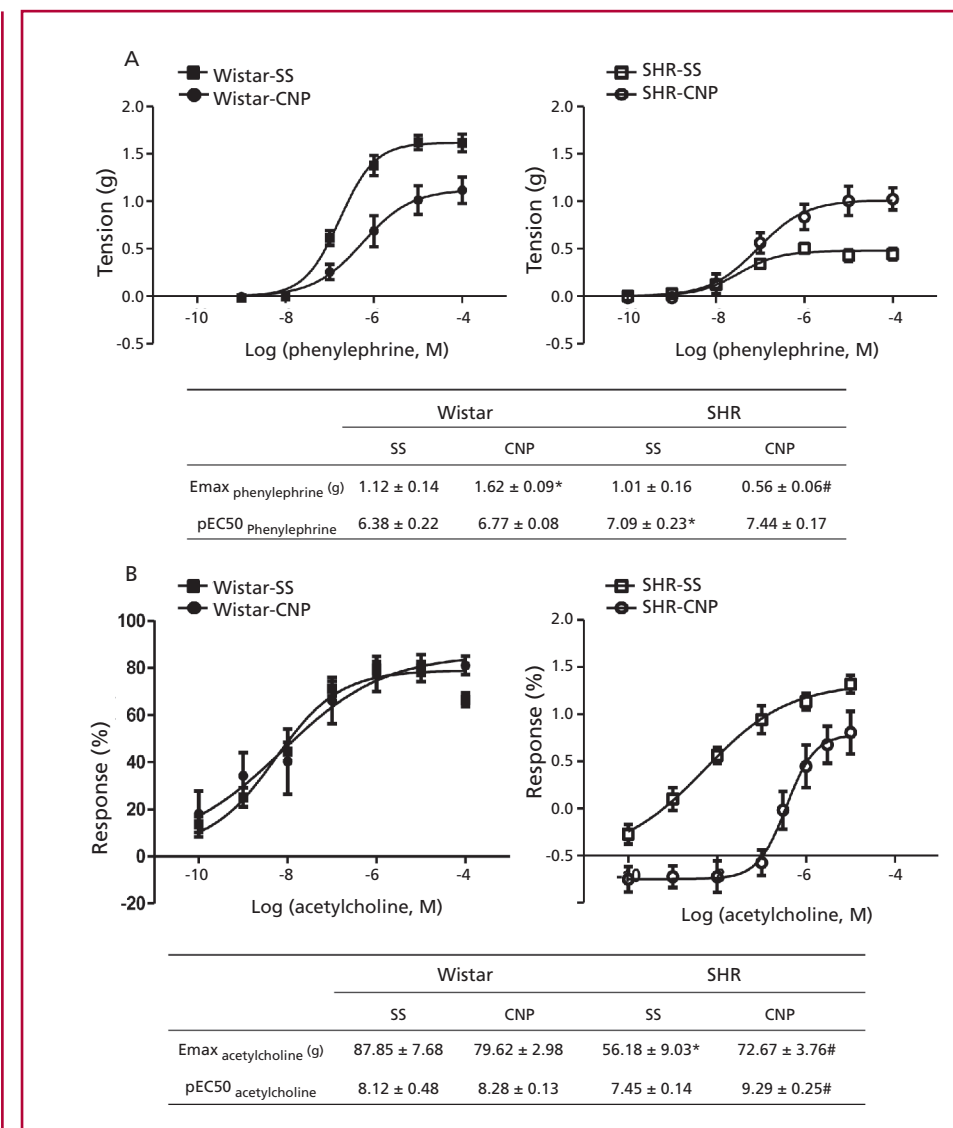
Table 1. Effect of chronic treatment with type-C natriuretic peptide on ventricular function in normotensive and spontaneously hypertensive rats

	Wistar		SHR	
	SS	CNP	SS	CNP
LV (g)	0.841 ± 0.041	0.889 ± 0.060	1.033 ± 0.057*	1.019 ± 0.035
Heart weight (g)	1.153 ± 0.040	1.204 ± 0.070	1.354 ± 0.049*	1.353 ± 0.025
LV/body weight (g/100 g)	0.229 ± 0.006	0.222 ± 0.006	0.294 ± 0.013*	0.300 ± 0.004

All the values are expressed using tibial length as growth parameter. SHR: Spontaneously hypertensive rats. SS: Saline solution. CNP: C-type natriuretic peptide. LV: Left ventricle. Statistical analysis: Analysis of variance (ANOVA), followed by Bonferroni post-hoc test for multiple comparisons. Results are expressed as mean±standard error of the mean, n = 6 rats/group. * p < 0.01 vs. Wistar-SS

Table 2. Effect of chronic treatment with type-C natriuretic peptide on left ventricular morphometric parameters in normotensive and spontaneously hypertensive rats

Fig. 2. Effect of 14-day chronic treatment with type-C natriuretic peptide (CNP) on vascular reactivity of the aorta in normotensive and spontaneously hypertensive rats (SHR). Panel A: Phenylephrine-induced vasoconstriction after phenylephrine increasing concentrations (10^{-10} M- 10^{-4} M) in Wistar rats and SHR. Panel B: Acetylcholine-induced vasodilation after acetylcholine increasing concentrations (10^{-10} M- 10^{-4} M) in Wistar rats and SHR. SS: Saline solution. Statistical analysis: Analysis of variance (ANOVA), followed by Bonferroni post-hoc test for multiple comparisons. Results are expressed as mean \pm standard error of the mean, n = 6 rats/group.



did not modify the vasodilator effect of acetylcholine in normotensive rats, this effect was induced in SHR, shifting the concentration-response curve to the left and increasing the maximal response to acetylcholine. In agreement with our results, in a recent study Li et al. have demonstrated that chronic infusion of a selective agonist of type-C natriuretic receptor increases the vasodilator response to carbachol in SHR but does not induce changes in normotensive Wistar-Kyoto rats. (19) Considering these results, the administration of exogenous CNP has a beneficial effect on BP regulation, evidenced in SHR by aortic changes to vasoconstrictor and vasodilator agents, favoring the latter and decreasing BP levels.

Moreover, while there was no difference in the echocardiogram of normotensive rats, CNP chronic treatment increased cardiac output in SHR. Although the duration of CNP treatment was not long enough to produce changes in LV contractility, the effects on the vascular tone and the SBP reduction in SHR re-

ceiving 14-day CNP treatment induced changes in LV loading conditions, which might reflect in long-term LV function improvement. In mice undergoing chronic treatment with angiotensin II, CNP could reverse the angiotensin II effect on cardiac hypertrophy and prevent reduction in shortening fraction observed in this model. (20) In addition, CNP inhibited hypertrophy markers in cardiomyocyte cultures; (21) however, although CNP treatment does not induce changes in LVMI in SHR, we cannot rule out that chronic administration of CNP promotes micro-structural changes which can explain greater EDV. This could be due to the fact that SHR have elevated levels of angiotensin II, plasma catecholamines and atrial and type-B natriuretic peptides, and lower availability of NO, among other abnormalities, making this model more complex compared with other models studied and preventing an antihypertrophic effect.

Our results showing that CNP could increase NO bioavailability inducing higher NOS activity; how-

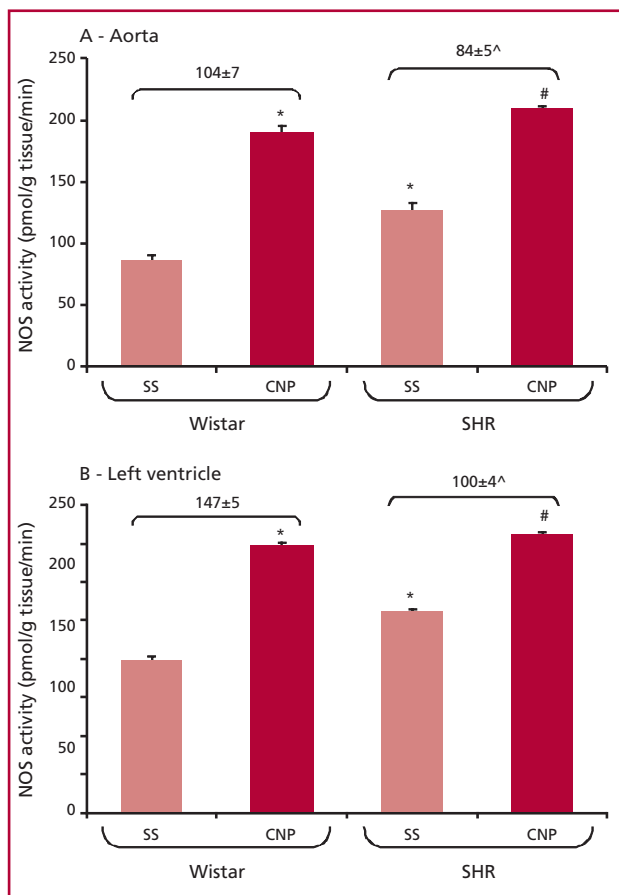


Fig. 3. Effect of 14-day chronic treatment with type-C natriuretic peptide (CNP) on nitric oxide synthase (NOS) activity in normotensive and spontaneously hypertensive rats (SHR). Panel A: NOS activity in the aorta of Wistar rats and SHR. Panel B: NOS activity in the left ventricle of Wistar rats and SHR. SS: Saline solution. Statistical analysis: Analysis of variance (ANOVA), followed by Bonferroni post-hoc test for multiple comparisons. Results are expressed as mean ± standard error of the mean, n = 6 rats/group. * p < 0.01 vs. Wistar-SS; # p < 0.01 vs. SHR-SS; ^Δ p < 0.01 vs. Wistar.

ever, NO availability could increase by reducing the production of reactive oxygen species. There is evidence of the antioxidant effect of CNP on certain tissues: in the heart, CNP reduces the production of reactive oxygen species induced by angiotensin II (20) and the activation of NPR-C reduces the expression of NADPH oxidase subunits, as Nox4 and p47^{phox} and NADPH oxidase activity in vascular smooth muscle cells, (19) inhibiting superoxide release and increasing NO bioavailability. (8) As SHR present an imbalanced oxidative state and higher levels of reactive oxygen species, (7) the anti-oxidant effect of CNP could explain the hypotensive effect seen in SHR despite having lower NOS activity.

Perspectives

Endogenous levels of natriuretic peptides are elevated in heart failure patients, and the body becomes re-

sistant to their vasodilator, diuretic and natriuretic effects.

However, infusion of exogenous natriuretic peptides transiently reverses this resistance.

The importance of CNP in the treatment of BP and its associated conditions has not been defined yet. The evaluation of the mechanisms at the molecular level in HTN models and their relation with other systems of BP regulation, as the NO system, may contribute to the development of new therapeutic strategies.

CONCLUSIONS

CNP induces cardiovascular and NO system changes which may be beneficial in this model of hypertension.

Conflicts of interest

None Declared

(See author's conflicts of interest forms in the web / Supplementary Material)

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