Spironolactone Differently Influences Remodeling of the Left Ventricle and Aorta in L-NAME-Induced Hypertension

Fedor Šimko[1,2], Jana Matúšková[1], Ivan Ľupták[1], Terézia Pinčíková[1], Kristína Krajčírovičová[1], Svetoslav Štvrtina[3], Július Pomšár[1], Václav Pelouch[4], Ľudovít Paulis[1,5], Oľga Pecháňová[5,6]

[1]Institute of Pathophysiology, [2]3rd Clinic of Medicine, and [3]Institute of Pathology, School of Medicine, Comenius University, Bratislava, Slovak Republic, [4]Department of Medical Chemistry and Biochemistry, 2nd Faculty of Medicine, Charles University and Center for Cardiovascular Research, Prague, Czech Republic, [5]Institute of Normal and Pathologic Physiology, Slovak Academy of Sciences, Bratislava, Slovak Republic, [6]Institute of Physiology, Academy of Sciences of the Czech Republic, Prague, Czech Republic

Running title: Spironolactone in the remodeling of heart and aorta

Address for correspondence: Prof. Fedor ŠIMKO, MD, PhD, Department of Pathophysiology, School of Medicine, Comenius University, Sasinkova 4, 81372 Bratislava, Slovak Republic, Tel.: +421-(0)2-59357276, Fax: +421-(0)2-59357601, E-mail: fedor.simko@fmed.uniba.sk

Summary

Aldosterone receptor antagonist, spironolactone, has been shown to prevent remodeling of the heart in several models of left ventricular hypertrophy. The aim of the present study was to determine whether treatment with spironolactone can prevent hypertension, reduction of tissue nitric oxide synthase activity and left ventricular (LV) and aortic remodeling in N^G-nitro–L-arginine methyl ester (L-NAME)-induced hypertension. Four groups of rats were investigated: control, spironolactone (200 mg/kg), L-NAME (40 mg/kg) and L-NAME + spironolactone (in corresponding doses). Animals were sacrificed and studied after 5 weeks of treatment. The decrease of NO-synthase activity in the LV and kidney was associated with the development of hypertension and hypertrophy of the left ventricle (LV), with increased DNA concentration in the LV, and remodeling of the aorta in the L-NAME group. Spironolactone prevented the inhibition of NO-synthase activity in the LV and kidney and in part prevented hypertension and LVH development and the increase in DNA concentration. However, remodeling of the aorta was not prevented by spironolactone treatment. We conclude that the aldosterone receptor antagonist spironolactone improved nitric oxide production and partially prevented hypertension and LVH development without preventing hypertrophy of the aorta in NO-deficient hypertension. The reactive growth of the heart and aorta seems to be controlled by different mechanisms in L-NAME-induced hypertension.

Key words: L-NAME, NO-deficient hypertension, spironolactone, left ventricular hypertrophy, nitric oxide, aorta remodeling

Introduction

Hypertrophy of the left ventricle (LVH), although an adaptive-compensatory mechanism, involves the risk of increased cardiovascular morbidity and mortality (Šimko 2002). It is generally believed that prevention or regression of pathologic remodeling of the heart and vessels diminish the cardiovascular risk. As a result, a number of drugs are being tested with the aim to disclose their potential in protecting the cardiovascular system against the consequences of the hemodynamically induced hypertrophic growth (Šimko 1994, 1996, Pecháňová et al. 2006, Šimko and Paulis 2007).

Although a relation of renin-angiotensin-aldosterone system is known to be tightly bound to the pathologic restructuralisation of the heart and vessels (Šimko and Šimko 1999, Funder 2001), much more is known about the blockade of angiotensin II production or its effect than on the role of aldosterone receptor antagonism in the protection against this deleterious process (Brilla and Weber 1992, Lijnen and Petrov 2000, Schmidt and Schmieder 2003, Kojšová *et al*. 2006). However, the impressively positive results of the Randomised Aldacton Evaluation Study (RALES) attracted an attention to aldosterone blockade (Pitt *et al.* 1999). Mortality reduction by 30% in severe heart failure patients was revealed when small dose aldosterone receptor antagonist spironolactone was added to standard treatment with ACE-inhibitors. The protective effect of spironolactone on left ventricular remodeling was considered to be the most important mechanism participating on the reduction of morbidity and mortality in the RALES trial (Zannad *et al.* 2000).

The model of N^G-nitro-L-arginine methyl ester (L-NAME) induced hypertension is characterised by decreased nitric oxide (NO) production, and concommitant LVH, fibrosis and deterioration of peripheral organs including aorta, kidney and brain (Bernátová *et al.* 1999, Pereira and Mandarim de-Lacerda 2001, Šimko *et al.* 2004,2005, Pecháňová et al.2006). There are data that myocardial structural changes in the L-NAME-induced model of hypertension may in part be determined by elevation of serum aldosterone via increased AT1 receptor number in the adrenal gland (Usui *et al.* 1998). Hence, we supposed that spironolactone could act beneficially within the model of NO-deficient hypertension. The hypothesis we tested, was whether spironolactone is able to prevent hypertension as well as modify the remodeling of the heart and aorta in the NO-deficient hypertension model.

Materials and methods

Animals and treatment

Male Wistar rats, 15 weeks old, were randomly divided into four groups (n = 8 in each group). The first group served as a control. In the second group, L-NAME (Sigma Chemical Co, Germany) was given 40 mg/kg/day. The third group received spironolactone (Gedeon Richter, Hungary) 200 mg/kg/day. The fourth group received simultaneously L-NAME (40mg/kg) and spironolactone 200 mg/kg/day. All the substances were given for 5 weeks. L-NAME was given in tap water and spironolacton was mixed as an emulsion and was applied via a gavage twice daily. Moreover, all other animals were gavaged with placebo twice daily so that the same handling conditions were preserved for all animals in the experiment. All animals were housed at a temperature of 22 - 24°C in individual cages and fed a regular pellet diet *ad libitum.*

The investigation conformed to the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No 8523, revised 1985).

The systolic blood pressure was measured by noninvasive tail-cuff plethysmography each week. After 5 weeks, the animals were sacrificed by decapitation, the body weight (BW), heart weight (HW), left ventricle weight (LVW) and right ventricle weight (RVW) were determined and the LVW/BW as well as RVW/BW ratio were calculated. Samples of the left ventricle were used for the determination of NO-synthase activity and DNA concentration. NO-synthase activity was determined also in the kidney.

Determination of deoxyribonucleic acid (DNA) concentration

DNA concentration was analysed according Sambrook *et al.* (1989).

Morphometry of aorta

Samples of aorta were put in an upright position on cellulose filter membranes to maintain a round shape. The tissues were fixed for 24 hours in 10% phosphate buffer formalin. Then they were processed in paraffin. Before evaluating the tissues serial 5 μm thick sections were stained with haematoxylin and eosin and by van Gieson´s staining. Morphometric evaluation was performed on an Nikon-119 (Japan) light microscope connected to high-resolution CCD camera (GKB, CC8706S, Taiwan) equipped with a two-dimensional image analyzer (Impor Pro, Kvant sro, Slovakia) as described elsewhere (Babál *et al.* 1997). Media + intima wall thickness (WT) of the aorta was measured with omission of the adventitia. After measuring the inner perimeter, the inner diameter (ID) was calculated. Finally the WT to ID ratio

(WT/ID) and cross-sectional area (CSA) was calculated (CSA = 3.1416 x ((ID/2+WT)2-(ID/2)2)). The WT was expressed in µm, the ID in mm and the CSA in mm^2.

Assay of NO-synthase activity

Total NO-synthase activity was determined in crude homogenates of tissues by measuring the formation of [^{3} H]–L-arginine (Amersham International plc, UK), as described by Bredt and Snyder (1990) with some modifications. Briefly 50µl of 10% homogenates were incubated in the presence of 50 nmol/l Tris-HCl, pH 7.4, 20 µmol/l [^{3}H]-L-arginine (specific activity 5 GBq/mmol, about 100 000 dpm/min) 30 nmol/l calmodulin, 1 mmol/l β-NADPH, 3 µmol/l BH_4 and 2 mmol/l Ca^{2+} in a total volume of 100 µl. After 10 min incubation at 37°C, the reaction was stopped by addition of 1 ml of 20 mmol/l HEPES buffer pH 5.5, containing 2 mmol/l EDTA, 2 mmol/l EGTA and 1 mmol/l L-citrulline. The samples were centrifuged at 10 000 g for 1 min at 4°C and the suppernatant was applied to 1 ml Dowex 50 WX-8 columns (Na^+ form). L-[^{3}H] Cit was eluted with 1 ml of water and measured by liquid scintillation counting. NO-synthase activity was expressed as picokatal per gram of protein (pkat.(g.protein)$^{-1}$).

Statistical analysis

The results are expressed as mean ± S.E.M. Differences were considered significant if the *P*-value was less than 0.05. For statistical analysis, one way analysis of variance (ANOVA) and the Bonferroni test were used.

Results

Cardiovascular parameters

After five weeks of treatment, SBP was 127 ± 3 mmHg in the control group. In the L-NAME group SBP increased by 41% ($P < 0.05$). In the L-NAME+ spironolactone group, SBP was higher than in controls by 22% ($P < 0.05$) but lower if compared to L-NAME group by 11% ($P < 0.05$) (Fig. 1).

After five weeks of experiment, the LW/BW ratio was 1.16 ± 0.03 mg/g w.w. in controls. In the L-NAME group, the ratio increased by 16% ($P<0.05$) ν control group and in the L-NAME + spironolactone group the LVW/BW ratio returned to the level of the control group and was lower if compared to L-NAME group by 14% ($P<0.05$) (Fig. 2A).

The RVW/BW ratio was not affected in any group (Table 1).

DNA concentration

The concentration of DNA was 593 ± 46 µg/gww in the left ventricle of the control group. In the L-NAME group, DNA concentration increased by 56% ($P<0.05$) ν control. In the L-NAME+spironolactone group, DNA concentration was reduced when compared to the L-NAME group by 21%($P<0.05$) (Fig. 2B).

Morphometry of aorta

The thickness of aorta (tunica intima + tunica media) was 102.9 ± 5.0 µm in the control group. In the L-NAME group, the thickness of aorta increased by 18% ($P<0.05$) ν control and the addition of spironolactone to L-NAME did not prevent the thickening of aorta compared to the L-NAME group (Fig. 3A).

The cross sectional area of aorta was 344 ± 54 μm^2 in the control group. In the L-NAME group, the cross sectional area was increased by 70% ($P < 0.05$) ν control and the addition of spironolactone to L-NAME did not prevent the enlargement of the cross sectional area of aorta compared to the L-NAME group (Fig. 3B).

NO-synthase activity

NO-synthase activity was 5.21 ± 0.32 pkat/g prot. in the left ventricle of the control group. In the L-NAME group, NO-synthase activity was inhibited by 40% ($P < 0.05$) ν control group and the addition of spironolactone to L-NAME returned the NOS activity to the control level. (Fig. 4A). In the kidney, NO-synthase activity was 16.40 ± 1.03 pkat/g prot in the control group. In the L-NAME group, NO-synthase activity was inhibited by 26% ($P < 0.05$) ν control and the addition of spironolactone to L-NAME returned NO-synthase activity on the level of the control group (Fig. 4B).

Discussion

The present study demonstrates the influence of the aldosterone receptor antagonist spironolactone on the growth of the heart and aorta in L-NAME induced hypertension. L-NAME caused a reduction of NO-synthase activity in the heart and kidney, an increase in systolic blood pressure, hypertrophy of the LV and aorta and DNA concentration in the LV. Simultaneous treatment with spironolactone normalised NO synthase activity in the heart and kidney, attenuated the increase in systolic blood pressure and prevented the development of LVH and the increase in DNA concentration in the LV. However, the hypertrophy of aorta remained unaffected.

Aldosterone has been shown to participate on the pathologic myocardial remodeling. Chronic elevation of aldosterone level together with increased sodium intake provoked myocardial fibrosis in the left and right ventricles, which involved the signs of both reactive and reparative fibrosis (Brilla and Weber 1992). Small, nondepressor doses of spironolactone prevented fibrosis of the LV without influencing salt-induced hypertension or LV hypertrophy (Brilla *et al.* 1993). On the other hand, higher, antihypertensive spironolactone dose also prevented LV hypertrophy development (Brilla *et al.* 1993). The reduction of morbidity and mortality in severe heart failure patients in the RALES trial with spironolactone (Pitt *et al*. 1999) or in patients with mild heart failure after myocardial infarction with eplerenone (Pitt *et al.* 2005) has been attributed to an antifibrotic effect of mineralocorticoid receptor blockade (Zannad *et al.* 2000). However, the mechanism of this cardiovascular protection of aldosterone antagonists remains only hypothetic. The inhibition of systemic and local aldosterone production, improved ionic composition in plasma, reduction of endothelin and stimulation of bradykinin pathway and/or inhibition of aldosterone-induced hemodynamic alterations and inflammatory reaction might be involved (Šimko *et al.* 2002, Schmidt and Schmieder 2003).

Hypertrophy of the LV in the L-NAME model of hypertension was shown to be linked to increased fibrosis (Pecháňová *et al.* 1997, Šimko *et al.* 2004, 2005) and protein remodeling of the left ventricle (Pecháňová *et al.* 1997, Bernátová *et al.* 2000). In our previous experiment, the ACE-inhibitor captopril, completely prevented LVH and fibrosis development. However, NO synthase activity remained inhibited in all organs investigated (LV, brain, kidney and aorta) (Bernátová *et al.* 1999). Similarly, in the same model of hypertension, imidapril prevented LVH and nephrosclerosis

development without improving the depressed nitrate/nitric production (Akuzawa *et al.* 1998). It was suggested that prevention of cardiovascular remodeling in the L-NAME model of hypertension was achieved by mechanisms different from the restoration of NO synthase activity (Bernátová *et al.* 1996, Bernátová 1999). On the other hand, spironolactone in this experiment improved NO synthase activity in both, LV and kidney, indicating improved NO production. Moreover, it has been shown previously in our laboratory that spironolactone increased both thiol and nitrosothiol groups in the kidney (Pecháňová *et al.* 2006). Both these effects can prolong NO half-life and potentiate its vasorelaxant effect (Stamler *et al.*1992, Ignaro *et al.* 1981). As a result, reduction of blood pressure in this experiment might have been related to vasodilatative effect of NO. Prevention of LVH and fibrosis (represented by increased DNA concentration) could be related, beside the hemodynamic relief, to the direct antiproliferative effect of nitric oxide. However, inhibitory action of spironolactone on the proliferative effect of tissue or plasmatic aldosterone could also participate (Schmidt and Schmieder 2003, Lijnen and Petrov 2000).

Our results are partly contradictory to data obtained by Pereira and Mandarim-de-Lacerda (2003). In the spontanously hypertensive rats (SHR) they demonstrated that although spironolactone reduced blood pressure, improved capillarisation of myocardial tissue and using high dose reduced fibrosis in the LV, spironolactone was not able to prevent the hypertrophy of cardiomyocytes. This difference could be explained in part by greater dose of spironolactone used in our experiment (200 mg/kg/day in our experiment vs. 5, 10 or 30 mg/kg/day in above mentioned experiment with SHR), since Brilla *et al*. (1993) have indeed shown that reduction of LVH by spironolactone is dose-dependent.

Most interestingly, despite prevention of LVH development, spironolactone, although administered in pharmacological dose, has not prevented the hypertrophic growth of the aorta. Similarly, in a regression experiment using the model of NO-deficient hypertension, spironolactone reversed LVH without changing hypertrophy of the aorta. It may be suggested that the growth of LV myocardium and aorta could be differently regulated. While the growth of the LV seems to be associated with nitric oxide or angiotensin - aldosterone pathway, the growth of aorta might be relatively independent from the above mentioned mechanisms. One can speculate that hypertrophy of aorta may be controlled by a proliferation stimulating humoral factors that are relatively uninfluenced by spironolactone, like endothelin or catecholamines. We conclude that spironolactone prevented pathological remodeling of the heart but not of the aorta in the L-NAME-induced hypertension despite improving NO-synthase activity in the heart, kidney and aorta. The growth of the LV and aorta seems to be differently controlled in the model of L-NAME-induced hypertension.

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References

AKUZAWA N, NAKAMURA T, KURASHINA T, SAITO Y, HOSHINO J, SAKAMOTO H, SUMINO H, ONO Z, NAGAI R: Antihypertensive agents prevent nephrosclerosis and left ventricular hypertrophy induced in rats by prolonged inhibition of nitric oxide synthesis. *Am J Hypertens* **11**: 697-707, 1998.

BABÁL P, PECHÁŇOVÁ O, BERNÁTOVÁ I, ŠTVRTINA S: Chronic inhibition of NO synthesis produces myocardial fibrosis and arterial media hyperplasia. *Histol Histopathol* **12**: 623-629, 1997.

BERNÁTOVÁ I, PECHÁŇOVÁ O, ŠIMKO F: Captopril prevents NO-deficient hypertension and left ventricular hypertrophy without affecting nitric oxide synthase activity in rats. *Physiol Res* **45**: 311-316, 1996.

BERNÁTOVÁ I, PECHÁŇOVÁ O, ŠIMKO F: Effect of captopril in L-NAME-induced hypertension on the rat myocardium, aorta, brain and kidney. *Exp Physiol* **84**: 1095-1105, 1999.

BERNÁTOVÁ I, PECHÁŇOVÁ O, PELOUCH V, ŠIMKO F: Regression of chronic L-NAME-treatment-induced left ventricular hypertrophy: effect of captopril. *J Mol Cell Cardiol* **32**: 177-185, 2000.

BREDT DS, SNYDER SH: Isolation of nitric oxide synthetase, a calmodulin-requiring enzyme. *Proc Natl Acad Sci USA* **87**: 682-685, 1990.

BRILLA CG, WEBER KT: Reactive and reparative myocardial fibrosis in arterial hypertension in the rat. *Cardiovasc Res* **26**: 671-677, 1992.

BRILLA CG, MATSUBARA LS, WEBER KT: Anti-aldosterone treatment and the prevention of myocardial fibrosis in primary and secondary hyperaldosteronism. *J Mol Cell Cardiol* **25**: 563-575, 1993.

FUNDER J: Mineralocorticoids and cardiac fibrosis: The decade in review. *Clin Exp Pharmacol Physiol* **28**: 1002-1006, 2001.

IGNARRO LI, LIPPTON H, EDWARDS JC, BARICOS WH, HYMAN AL, KADOWITZ PJ, GRUETER CA: Mechanism of vascular smooth muscle relaxation by organic nitrates, nitrites, nitroprusside and nitric oxide: evidence for the involvement of S-nitrosothiols as active intermediates. *J Pharmacol Exp Ther* **218**: 739-749, 1981.

KOJŠOVÁ S, JENDEKOVÁ L, ZICHA J, KUNEŠ, J, ANDRIANTSITOHAJNA R, PECHÁŇOVÁ O: The effect of different antioxidants on nitric oxide production in hypertensive rats (minireview). *Physiol Re*s **55** (Suppl 1): S13-S16, 2006.

LIJNEN P, PETROV V: Induction of cardiac fibrosis by aldosterone. *J Mol Cell Cardiol* 2000; **32**: 865-879.

MANDARIM-DE-LACERDA CA, PEREIRA LM: The effect of spironolactone monotherapy on blood pressure and myocardial remodeling in spontaneously hypertensive rats: a stereological study. *J Biomed Sci* **10**: 50-57, 2003.

PECHÁŇOVÁ O, BERNÁTOVÁ I, PELOUCH V, ŠIMKO F: Protein remodeling of the heart in NO-deficient hypertension: the effect of captopril. *J Mol Cell Cardiol* **29**: 3365-3374, 1997.

PECHÁŇOVÁ O, REZZANI R, BABÁL P, BERNÁTOVÁ I, ANDRIANTSITOHAJNA R: Beneficial effects of provinols^{TM} : cardiovascular system and kidney (minireview). *Physiol Re*s **55** (Suppl 1): S17-S30, 2006a.

PECHÁŇOVÁ O, MATÚŠKOVÁ J, CAPÍKOVA D, JENDEKOVÁ L, PAULIS Ľ, ŠIMKO F: Effect of spironolaktone and captropril on nitric oxide and S-nitrosothiol formation in kidney of L-NAME-treated rats. *Kidney Int* **70**: 170-176, 2006b.

PEREIRA LM, MANDARIM-DE-LACERDA CA: Stereology of cardiac hypertrophy induced by NO blockade in rats treated with enalapril and verapamil. *Anal Quant Cytol Histol* **23**: 330-338, 2001.

PITT B, ZANNAD D F, REMME WJ, CODY R, CASTAIGNE A, PEREZ A, PALENSKY J, WITTES J: for the Randomized Aldactone Evaluation Study Investigators. The effect of spironolactone on morbidity and mortality in patients with severe heart failure. *N Engl J Med* **341**: 709-717, 1999.

PITT B, WHITE H, NICOLAU J, MARTINEZ F, GHEORGHIADE M, ASCHERMANN M, VAN VELDHUISEN DL, ZANNAD F, KRUM H, MUKHERJEE R, VINCENT J, for the EPHESUS Investigators: Eplerenone reduces mortality 30 days after randomization following acute myocardial infarction in patients with left ventricular systolic dysfunction and heart failure. *J Am Coll Cardiol* **46**: 425-431, 2005.

SAMBROOK J, FRITSCH EF, MANIATIS T: Isolation of DNA from mammalian cells. In: Ford N (ed). Molecular cloning. A laboratory manual. Second edition*. New Yourk: Cold Spring Harbor Laboratory Press* **9**: 9.16-9.19 , 1989.

SCHMIDT BMV, SCHMIEDER RE: Aldosterone- induced cardiac damage: focus on blood pressure independent effects. *Am J Hypertens* **16**: 80-86, 2003.

ŠIMKO F: Pathophysiological principles of the relation between myocardial hypertrophy of the left ventricle and its regression (minireview). Physiol Res **43**: 259-266, 1994.

ŠIMKO F: Left ventricular hypertrophy regression as process with variable biological implications (review). *Can J Cardiol* **12**: 507-51, 1996.

ŠIMKO F: Physiologic and pathologic myocardial hypertrophy: physiologic and pathologic regression of hypertrophy? *Med Hypotheses* **58**: 11-14, 2002.

ŠIMKO F: Statins – a prespective fot left ventricular hypertrophy treatment (review). *Eur J Clin Invest* **37**:681-691, 2007.

ŠIMKO F, PAULIS Ľ: Melatonin as a potential antihypertensive treatment (minireview). *J Pin Res* **42**: 319-322, 2007.

ŠIMKO F, ŠIMKO J: Heart failure and angiotensin converting enzyme inhibition: problems and perspectives (minireview). *Physiol Res* **48**: 1-8, 1999.

ŠIMKO F, ŠIMKO J: The potential role of nitric oxide in the hypertrophic growth of the left ventricle (minireview). *Physiol Res* **49**: 37-46, 2000.

ŠIMKO F, BADA V, ŠIMKOVÁ M, ŠIMKO J, KOVÁCS L, HULÍN I: The significance of aldosterone in chronic heart failure: the Rales Study (review). *Vnit Lék* **48**: 767-772, 2002.

ŠIMKO F, MATÚŠKOVÁ J, LUPTÁK I, KRAJČÍROVIČOVÁ K, KUCHARSKÁ J, GVOZDJÁKOVÁ A, BABÁL P, PECHÁŇOVÁ O: Effect of simvastatin on remodeling of the left ventricle and aorta in L-NAME-induced hypertension. *Life Sci* **74**: 1211-1224, 2004.

ŠIMKO F, LUPTÁK I, MATÚŠKOVÁ J, KRAJČÍROVIČOVÁ K, SUMBALOVÁ Z, KUCHARSKÁ J, GVOZDJÁKOVÁ A, ŠIMKO J, BABÁL P, PECHÁŇOVÁ O, BERNÁTOVÁ I: L-arginine fails to protect against myocardial remodeling in L-NAME-induced hypertension. *Eur J Clin Invest* **35**: 362-368, 2005.

STAMLER JS, JARAKI O, OSBORNE J, SIMON DI, KEANEY J, VITA J, SINGEL D, VALERI CR, LOSCALZO J: Nitric oxide circulates in mammalian plasma primarily as an S-nitroso adduct of serum albumin. *Proc Natl Acad Sci* USA **89**: 7674-7677, 1992.

USUI M, ICHIKI T, KATOH M, EGASHIRA K, TAKESHITA A: Regulation of angiotensin II receptor expression by nitric oxide in rat adrenal gland. *Hypertension* **32**: 527-533, 1998.

ZANNAD F, ALLA F, DOUSSET B, PEREZ A, PITT B: Limitation of excessive extracellular matrix turnover may contribute to survival benefit of spironolactone therapy in patients with congestive heart failure: insights from the randomized aldactone evalution study (RALES). Rales Investigators. *Circulation* **102**: 2700-2706, 2000.

Tables

Table 1 Effect of spironolactone and/or L-NAME treatment on the body weight (BW), left ventricle weight (LVW), right ventricle weight (RVW) and right ventricle to body weight ratio (RVW/BW) in NO-deficient rats

	Control	Spironolactone	L-NAME	L-NAME + Spironolactone
RVW (mg)	165 ± 5.9	146 ± 5.2*	171 ± 3.9	131 ± 3.8*$^{+}$
LVW (mg)	414 ± 16.4	354 ± 18.9	451 ± 15.3	365 ± 18.4^{+}
Body weight (g)	355 ± 8.6	345 ± 14.4	339 ± 11.7	317 ± 15.1
RVW/BW (mg/g)	0.46 ± 0.02	0.43 ± 0.01	0.50 ± 0.02	0.42 ± 0.02^{+}

Values are means ± SEM, ANOVA-Bonferroni: *P<0.05 compared to control, $^{+}$P<0.05 compared to L-NAME

Figure captions

Figure 1 Effect of 5-week L-NAME, spironolactone and L-NAME + spironolactone treatment on systolic blood pressure. *P<0.05 compared to control: $^{+}$P<0.05 compared to L-NAME group

Figure 2 Effect of 5-week L-NAME, spironolactone and L-NAME + spironolactone treatment on the LVW/BW ratio (A) and DNA concentration in the LV (B). *P<0.05 compared to control: $^{+}$P<0.05 compared to L-NAME group

Figure 3 Effect of 5-week L-NAME, spironolactone and L-NAME + spironolactone treatment on the thickness (A) and cross section area of the aorta (B). *P<0.05 compared to control: $^{+}$P<0.05 compared to L-NAME group

Figure 4 Effect of 5-week L-NAME, spironolactone and L-NAME + spironolactone treatment on NO-synthase activity in the left ventricle (A) and kidney (B). *P<0.05 compared to control: $^{+}$P<0.05 compared to L-NAME group

Figure 1

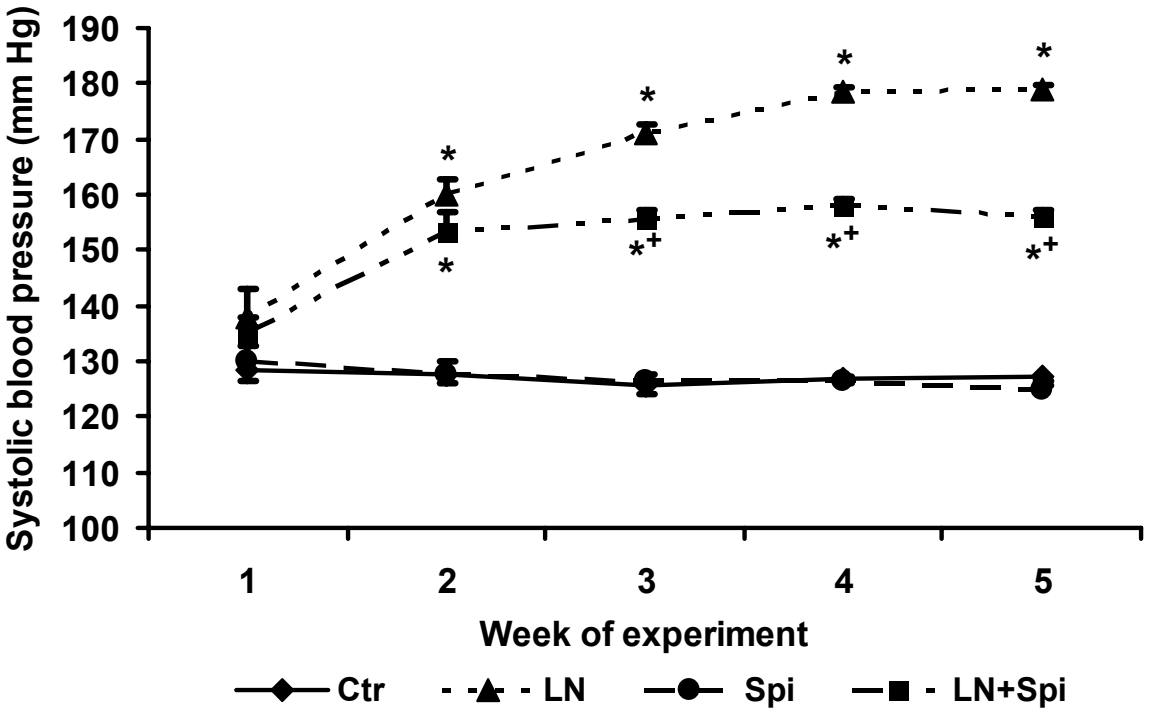
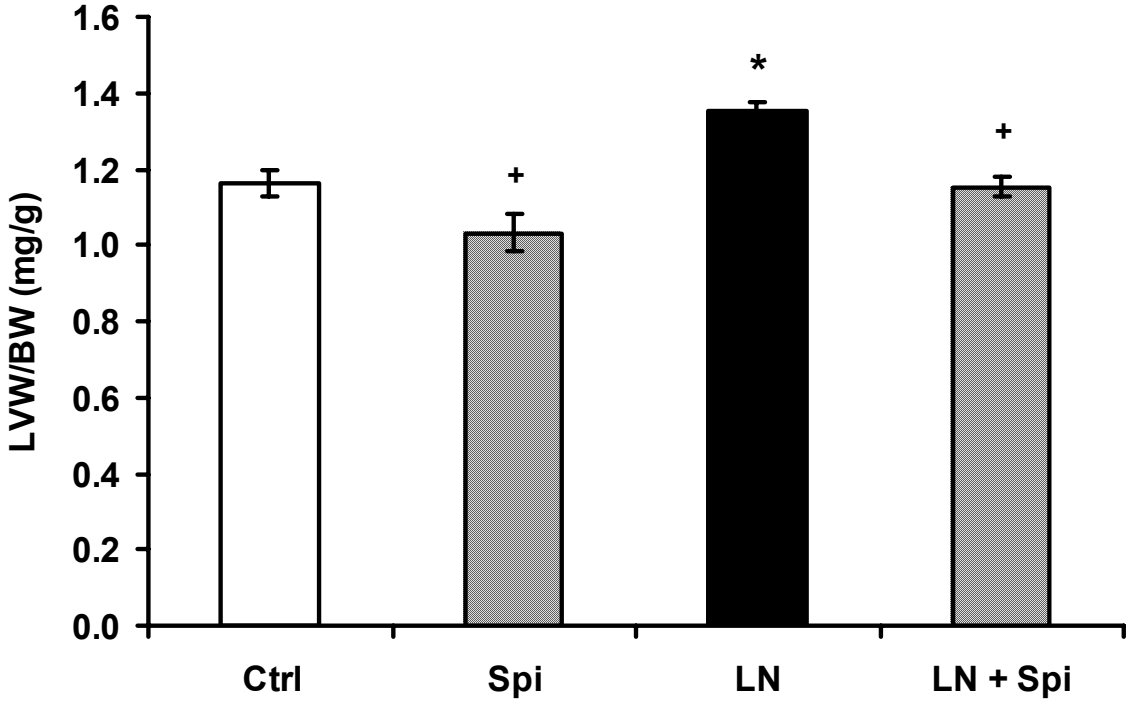

Systolic blood pressure (mm Hg)
190
180
170
160
150
140
130
120
110
100
1
2
3
4
5
Week of experiment
Ctr
LN
Spi
LN+Spi

Figure 2

A


LVW/BW (mg/g)
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
*
+
+
Ctrl
Spi
LN
LN + Spi

B

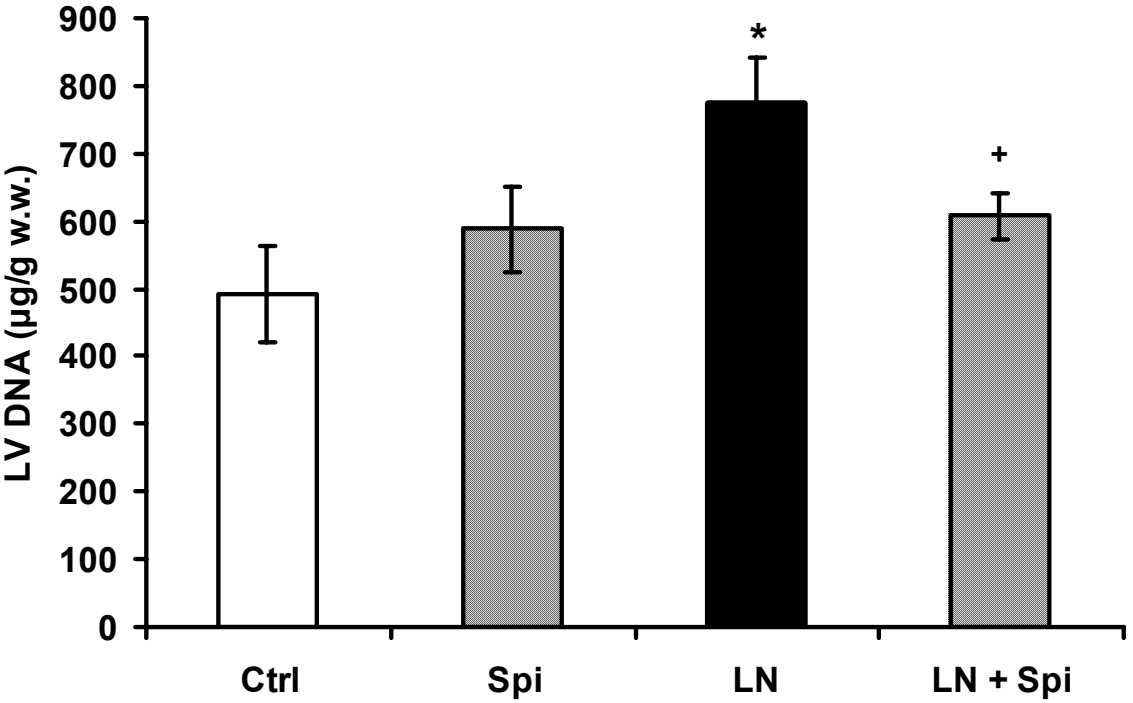
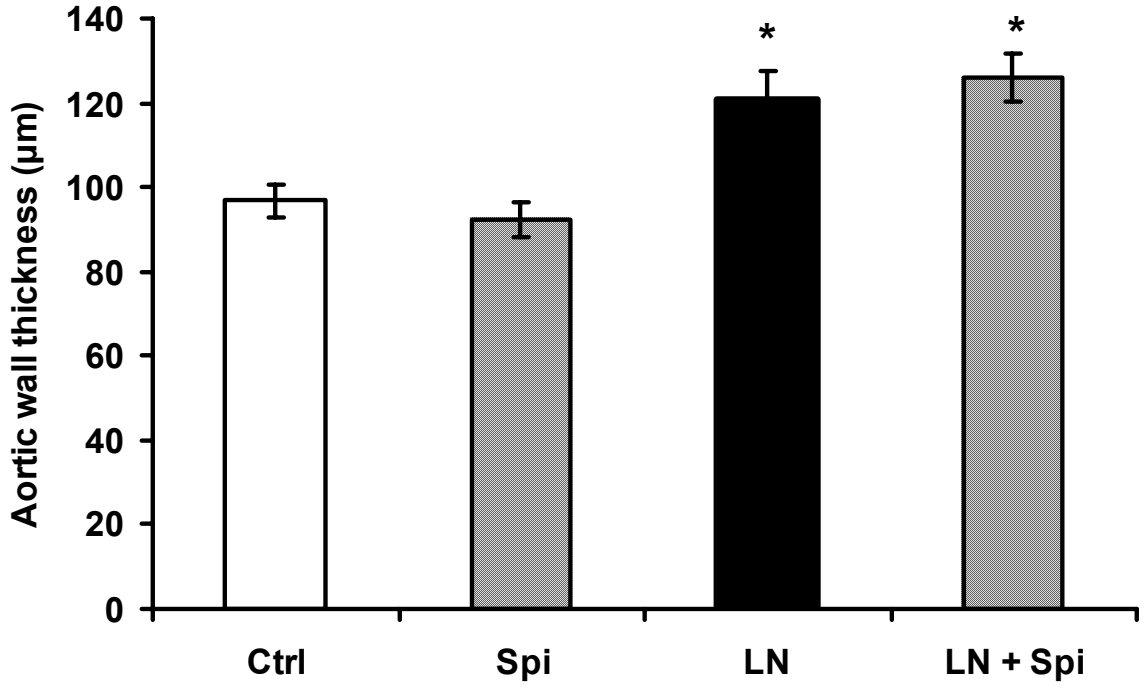

LV DNA (µg/g w.w.)
900
800
700
600
500
400
300
200
100
0
*
+
Ctrl
Spi
LN
LN + Spi

Figure 3

A


Aortic wall thickness (µm)
140
120
100
80
60
40
20
0
*
*
Ctrl
Spi
LN
LN + Spi

B

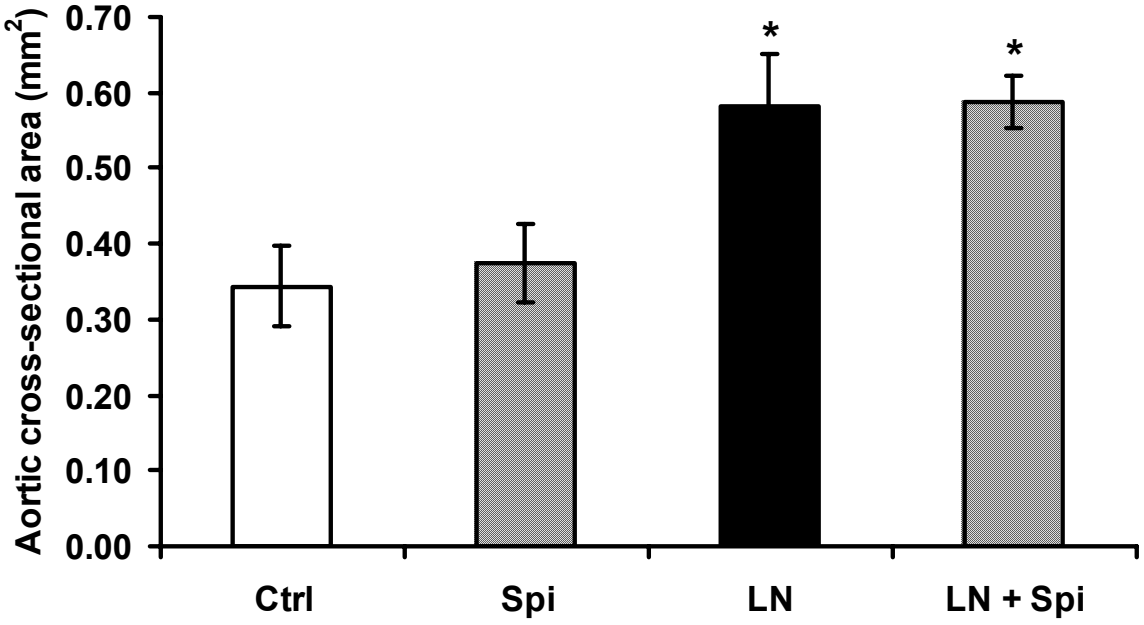
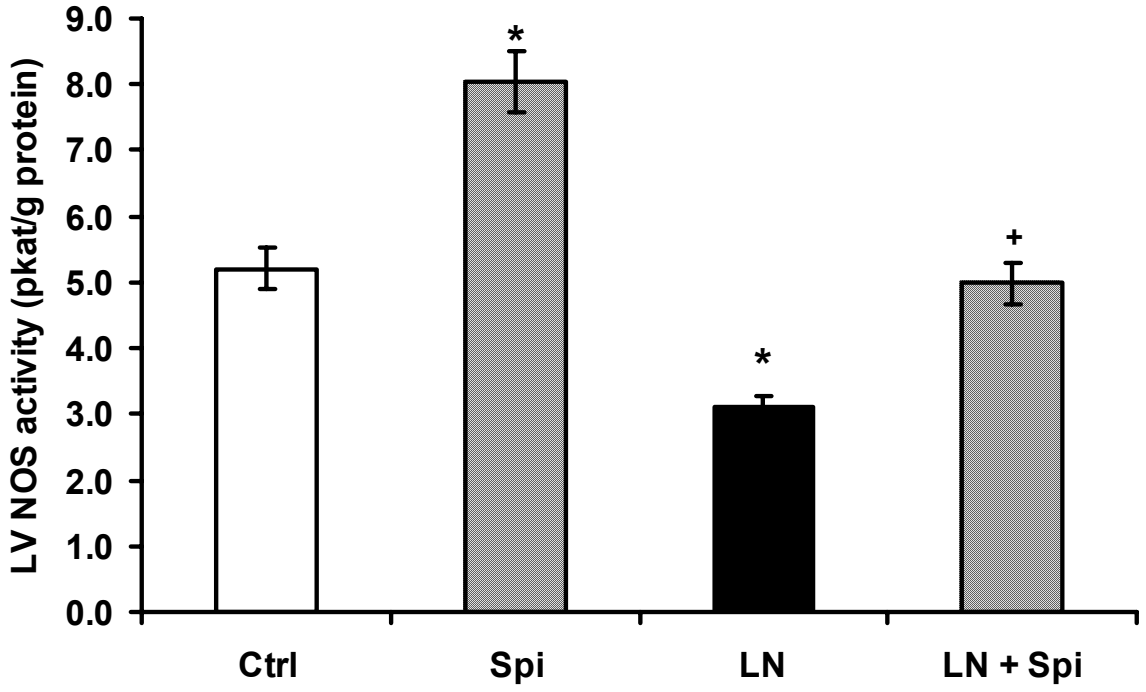
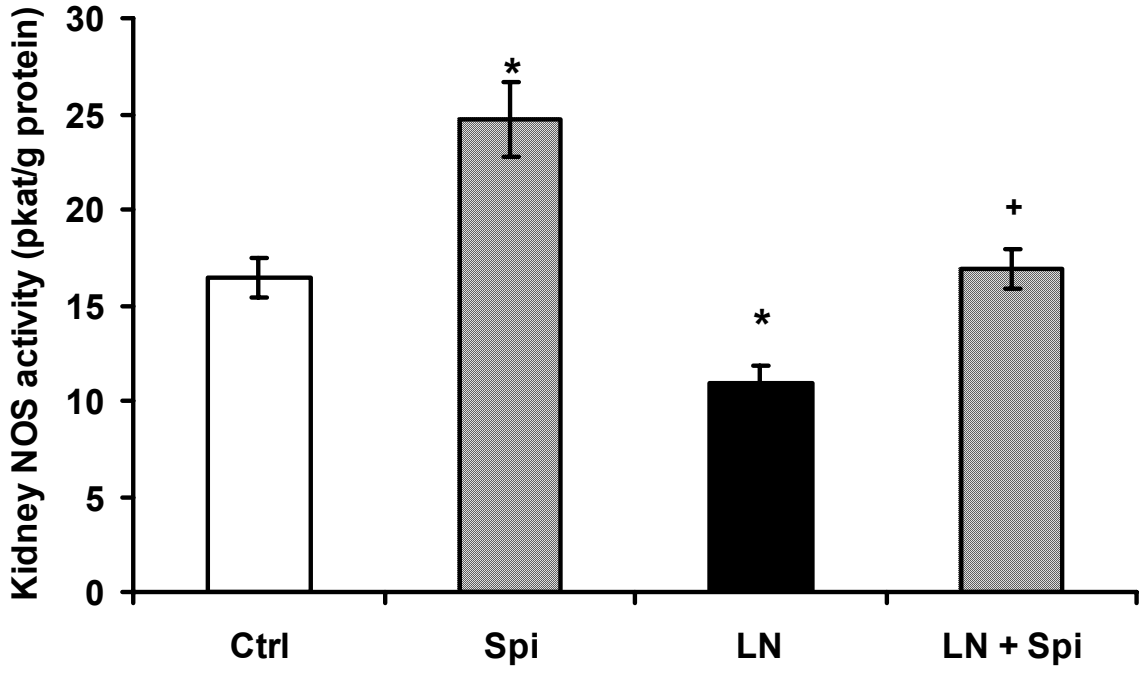

Aortic cross-sectional area (mm²)
0.70
0.60
0.50
0.40
0.30
0.20
0.10
0.00
*
*
Ctrl
Spi
LN
LN + Spi

Figure 4

A


LV NOS activity (pkat/g protein)
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
*
*
+
Ctrl
Spi
LN
LN + Spi

B


Kidney NOS activity (pkat/g protein)
30
25
20
15
10
5
0
*
*
+
Ctrl
Spi
LN
LN + Spi