ORIGINAL ARTICLE

Changes in Systolic and Diastolic Function in a Mouse Model Overexpressing Cardiac Angiotensin II AT-1 Receptor

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ABSTRACT

Angiotensin II (Ang II) is involved in various pathophysiological processes through the activation of angiotensin II AT-1 receptors. The purpose of this study was to assess in vivo and in vitro systolic and diastolic ventricular function in mice overexpressing the cardiac-specific Ang II AT-1 receptor (AT1R). A second objective was to determine whether ventricular function changes could be reversed with acute and chronic ATIR blockade.

Mice were divided into four experimental groups. The first group included non-transgenic animals (NTG, n=10), the second group consisted of transgenic mice (TG, n=7) with cardiac-specific AT1R overexpression and the third and fourth groups were TG animals treated with losartan (L) for 7 (TG L7, n=9) and 30 days (TG L30, n=7), respectively. Transgenic animals exhibited left ventricular hypertrophy (LVH) which was only regressed with losartan treatment for 30 days. They also presented a significant decrease in shortening fraction from 47.1±2.3% to 32.3±1.3% ($p < 0.05$) and of $+dP/dt_{max}$ from 7073 ± 674 to 3897.5 ± 209.7 mmHg/sec ($p < 0.05$). Systolic dysfunction recovered both with losartan treatment for 7 and 30 days.

Isovolumic relaxation time and t1/2 were 24.1±1.3 and 5.1±0.5ms, respectively, in the NTG group. These indexes increased to 33.1±2.2 and 8.4±0.4 ms, respectively, in TG mice ($p<0.05$). Diastolic dysfunction was completely reversed by losartan treatment for 7 and 30 days. In vitro ventricular function with controlled variables confirmed in vivo findings.

In conclusion, cardiac-specific AT1R overexpression induces systolic and diastolic ventricular dysfunction which is completely reversed by AT1R blockade. This beneficial effect is independent of left ventricular mass changes.

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Key words > Angiotensin II - Ventricular Function - AT-1 Receptor

Abbreviations >

ACE	Angiotensin converting enzyme	**LVSP**	Left ventricular systolic pressure
Ang II	Angiotensin II	**LVW**	Left ventricular weight
AT1R	Angiotensin AT-1 receptor	**NTG**	Non-transgenic
EF	Ejection fraction	**PKC**	Protein kinase C
IRT	Isovolumic relaxation time	**RAS**	Renin-angiotensin system
LVDP	Left ventricular developed pressure	**RVW**	Right ventricular weight
LVEDP	Left ventricular end-diastolic pressure	**TG**	Transgenic
LVH	Left ventricular hypertrophy	**TL**	Tibial length
LVPWTd	Left ventricular end-diastolic posterior wall thickness		

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INTRODUCTION

The rennin-angiotensin system (RAS) plays an important role in various physiological processes. However, under certain pathological conditions, its activity is increased causing deleterious effects. In response to pressure overload, angiotensin II (Ang II) promotes the development of cardiac hypertrophy, fibrosis and apoptosis. (1) Moreover, experimental studies both in animals (2) and patients (3) have shown that Ang II is relevant in the progression to heart failure. This finding has been confirmed and extended by studies in transgenic animals, showing that the progression to heart failure depends on the action of Ang II rather than on the presence of cardiac hypertrophy per se. (4,5) Consequently, the effects of Ang II on ventricular function are relevant and perhaps more significant than their properties to stimulate hypertrophy. Different studies conducted to investigate the effects of Ang II on ventricular function have shown controversial results, since some authors (6) found a negative inotropic response to acute infusion of Ang II, while others found positive inotropic effects in isolated heart preparations. (7, 8, 9, 10) Interestingly, Liang et al. (11) showed that Ang II has both positive and negative inotropic effects in isolated mouse hearts.

In order to clarify these results, transgenic models overexpressing different RAS components have been used. In this sense, Rivard et al. (12) showed that contractility is reduced in mice with cardiac overexpression of angiotensin AT-1 receptors (AT1R). In addition, these mice exhibit cardiac hypertrophy, arrhythmias, ventricular dysfunction, heart failure and increased mortality. (1, 13, 14) However, it is unclear whether changes in diastolic function are a direct consequence of AT1R overexpression or secondary to cardiac hypertrophy.

The aim of this study was thus to evaluate in vivo ventricular systolic and diastolic function in mice with cardiac-specific AT1R overexpression. Since contractility in vivo models can be modified by different variables (heart rate, loading conditions, etc.), ventricular function was also assessed in vitro, in an isolated heart model. A second objective was to determine whether acute and chronic AT1R receptor blockade reverses the changes in ventricular function.

METHODS

The experimental protocol was approved by the Institutional Animal Care and Use Committee of the School of Medicine, Universidad de Buenos Aires (IACUC Reg. CD #959/12).

Sixty-day old male mice weighing 24.7 ± 0.9 g were used. The transgenic model, overexpressing the cardiac-specific AT1R was generated from a FVB mice strain. These mice were designed using the α-myosin heavy chain as promoter to obtain the previously described cardiac-specific expression. (14) Mice were housed under a12 h:12 h light / darkness regimen at constant temperature (20-22°C) and with ad libitum access to food and water.

Animals were divided into four experimental groups. The first group included non-transgenic animals (NTG, n = 10); the second group consisted of transgenic animals (TG, n = 7), and the third and fourth groups were transgenic animals treated with losartan (L) 50 mg/kg/day for 7 (TG L7, n=9) and 30 days (TG L30, n=7), respectively.

In vivo ventricular function

In vivo ventricular function analysis was performed using two methods: echocardiography and cardiac catheterization.

Echocardiography

Mice were anesthetized with 290 mg/kg of 2.5% Avertin solution (Sigma-Aldrich), and the echocardiography was performed with an Acuson Sequoia C512 system equipped with a 14 MHz lineal ultrasound transducer. Left ventricular end-diastolic and end-systolic diameters were acquired in 2D mode. Ejection fraction (EF) and shortening fraction were calculated and both ejective indexes were used to assess systolic function.

Evaluation of left ventricular diastolic function was made from the E and A waves, the E/A ratio and the isovolumic relaxation time (IRT).

Cardiac catheterization

Animals were anesthetized with 290 mg/kg of 2.5% Avertin solution (Sigma-Aldrich). The jugular vein was dissected free and a silastic catheter (1 French) was inserted into the vein for drug infusion. Then, the right carotid artery was dissected free and a second catheter (Millar® 1.4 French) was inserted into the artery. This catheter was advanced into the left ventricle to measure left ventricular systolic pressure (LVSP, mmHg) and left ventricular end diastolic pressure (LVEDP, mmHg). In addition, $+dP/dt_{max}$ (mmHg/s) and t1/2 (time required for left ventricular pressure to decrease to 50% of its value at dP/dt^{min}) were calculated.

In vitro ventricular function

Animals from the NTG, TG and TG treated with losartan for 7 days (LG 7L) groups were sacrificed with pentobarbital sodium (150 mg/kg). Then, the heart was rapidly excised and mounted by the aortic root in an isolated perfusion system according to the Langendorff technique. Hearts were perfused with Krebs-Henseleit buffer containing: NaCl 118.5 mM, KCl 4.7 mM, NaHCO3 24.8 mM, KH2PO4 1,2 mM, MgSO4 1.2 mM, CaCl2 2.5 mM and glucose 10 mM, at pH 7.2-7-4. Temperature was maintained at 37°C and the buffer was bubbled with carbogen (95% O2-5% CO2). Two electrodes were connected to a pacemaker to keep a constant heart rate of 450 beats per minute.

A latex balloon was inserted through the left atrium into the left ventricle and connected to a tube whose distal end was attached to a pressure transducer (Deltram II, Utah Medical System). The balloon volume was adjusted to obtain diastolic pressure between 8 and 10 mmHg. Coronary perfusion pressure (CPP) was also recorded using a pressure transducer connected to the perfusion line. Coronary flow was adjusted to a CPP of 70.5 ± 4.2 mmHg and kept constant throughout the experiment.

Finally, left ventricular developed pressure (LVDP) was calculated as the difference between peak systolic and end-diastolic pressures.

Drug administration

Losartan (50 mg/kg/day) was administered in the drinking water for 7 and 30 days, starting 30 days after birth.

Morphology

Animals were sacrificed with an overdose of pentobarbital (150 mg/kg). The cardiopulmonary block was excised and the left and right atria and ventricles were dissected free and weighed (RVW and LVW, respectively). Body weight (BW) and tibial length (TL) were recorded and the corresponding LVW/BW, LVW/TL, RVW/BW and RVW/TL were calculated.

Statistical analysis

Data were expressed as mean ± standard error. One-way analysis of variance (ANOVA) was used to compare groups followed by Student´s t test, with Bonferroni´s correction to adjust the p value for multiple comparisons. A p value < 0.05/k, where k represents the number of comparisons, was considered statistically significant.

RESULTS

There was a significant increase of LVW/BW and LVW/TL in the TG group, indicating presence of left ventricular hypertrophy (LVH) (Table 1). Losartan treatment for 7 days (TG 7L) did not revert LVH, a result that was obtained when the treatment was extended to 30 days (TG L30). The same results were observed in the echocardiographic assessment of left ventricular end-diastolic posterior wall thickness (LVPWTd) (Table2). An important aspect is that the TG group exhibited lower heart rate than NTG animals, a finding that was not regressed by losartan. Moreover, lung weight did not show significant differences among groups (data not shown).

In vivo ventricular function

In vivo ventricular function assessment is illustrated in Figure 1. AT1R overexpression produced systolic dysfunction evidenced by a significant decrease of shortening fraction from 47.1 ± 2.3 to 32.3 ± 1.3% ($p< 0.05$) and of $+dP/dt_{max}$ from 7073 ± 674 to 3897.5 ± 209.7 mmHg/s ($p< 0.05$) (Figure 1, panels A and B). Mice receiving losartan treatment for 7 days showed a trend to increase shortening fraction though this result was not statistically significant. After 30-day treatment, this index recovered to 42.2 ± 2.4%. A similar behavior was seen for $+dP/dt_{max}$ which recovered to 5623 ± 154 mmHg/s with losartan for 7 days ($p < 0.05$), and to 6554 ± 619 mmHg/s with losartan for 30 days.

Diastolic function was evaluated by Doppler echocardiography (IRT and t1/2, calculated from the left ventricular pressure curve (Figure 2, panels A and B). In NTG mice, IRT was 24.1 ± 1.3 ms and increased to 33.1 ± 2.2 ms in TG animals ($p < 0.05$). The same behavior was observed for t1/2, which increased from 5.1 ± 0.5 ms in the NTG group to 8.4 ± 0.4 ms in TG animals ($p < 0.05$). This change in diastolic function was completely reversed by losartan treatment for 7 and 30 days, respectively.

Figure 3 shows that LVEDP in NTG mice was 3.1 ± 0.2 mmHg and increased to 6.3 ± 0.7 mmHg in the TG group ($p < 0.05$). This increase was fully reversed by losartan treatment for 7 or 30 days.

Table 1. Morphometric variables

	NTG	TG	TG L7	TG L30
BW, g	23.0±1.6	23.6±0.8	26±1.3	25.3±0.3
HW, mg	103.8±4.2	124.2±3.6*	136.3±5.2*	110.8±1.8#
LVW, mg	74.9±5.1	90.1±5.6*	96.6±2.8*	83.8±0.6#
TL, mm	16.4±0.5	16.4±0.4	16.6±0.5	17.6±0.1
LVW/BW, mg/g	3.3±0.1	4.0±0.2*	3.7±0.0*	3.1±0.5#
LVW/TL, mg/mm	4.6±0.1	5.2±0.1*	5.8±0.1*	3.8±0.0#
RVW/BW, mg/g	0.9±0.0	1.3±0.1*	1.2±0.0*	1.0±0.0#
RVW/TL, mg/mm	1.3±0.0	1.6±0.0*	1.9±0.0*	1.6±0.1

NTG: Non-transgenic animals; TG: Transgenic animals; TG L7: Transgenic animals treated with losartan for 7 days; TG L30: Transgenic animals treated with losartan por 30 days; BW: Body weight.; HW: Heart weight; LVW: Left ventricular weight. RVW: Right ventricular weight. TL: Tibial length. *p<0.05 vs NTG; # p<0.05 vs TG and TG L7.

Table 2. Echocardiographic indexes

	NTG	TG	TG L7	TG L30
LVPWTd(mm)	0.78±0.04	1.20±0.07*	1.13±0.05*	0.90±0.09#
LVEDD (mm)	3.62±0.19	3.72±0.13	3.78±0.23	3.91±0.18
LV LVESD (mm)	1.90±0.09	2.45±0.11	2.36±0.22	2.27±0.17
HR (bpm)	379±19	292±15*	260±9*	270±24*

NTG: Non-transgenic animals; TG: Transgenic animals; TG L7: Transgenic animals treated with losartan for 7 days; TG L30: Transgenic animals treated with losartan por 30 days; LVPWTd: Left ventricular diastolic posterior wall thickness . LVEDD: Left ventricular end-diastolic diameter. LVESD: Left ventricular end-systolic diameter. HR: Heart rate. *p<0.05 vs NTG; # p<0.05 vs TG.

In vitro ventricular function

Figure 4 illustrates the analysis of systolic (LVDP, mmHg) and diastolic (t1/2, ms) ventricular function in an isolated heart model at constant heart rate. As in the in vivo model, TG animals showed systolic dysfunction, independently of heart rate changes (Panel A). Interestingly, this alteration in the contractile state was regressed by losartan treatment for 7 days without modifying ventricular mass. Assessment of the diastolic component showed a similar behavior. A significant increase of t1/2 was observed in TG animals, which was completely reversed by losartan treatment for 7 days (Panel B).

DISCUSSION

The present study has shown that AT1R overexpression is associated with the presence of LVH and systolic and diastolic function impairment. Treatment with an AT1R blocker (losartan) improves ventricular function independently of ventricular hypertrophy.

Several authors (13, 14, 15) have shown that cardiac overexpression of AT1R is responsible for systolic ventricular dysfunction and sudden death. In this sense Hein et al. (13) and Zhai et al. (14) have shown that AT1R overexpression in mice favors a ventricular hypertrophy phenotype, cardiac arrhythmias and reduced heart rate. In a similar model, Paradis et al. (1) showed the presence of a similar morphological pattern, with ventricular hypertrophy and signs of heart failure. In this study, mice with cardiac overexpression of AT1R presented cardiac hypertrophy, significant decrease in heart rate and no signs of heart failure.

It is known that RAS has the ability to modulate ventricular function. (13) In this regard, Xiao et al. (16) demonstrated the presence of cardiac hypertrophy with preserved ventricular function in a mouse model with angiotensin converting enzyme (ACE) overexpression and elevated levels of Ang II. In contrast, the present study showed evidence of cardiac hypertrophy associated with systolic and diastolic dysfunction in TG mice. The discrepancy between these findings could be ascribed to different experimental models. It is also important to notice that models with ACE or AT1R overexpression have important differences, since increased cardiac Ang II could activate AT2R, whose role is not fully elucidated. Similarly, the increased expression of ACE in TG animals could regulate the concentration of other peptides such as bradykinin and N-acetyl-seryl-aspartyl-lysyl-proline (AcSDKP) producing different effects. Our findings

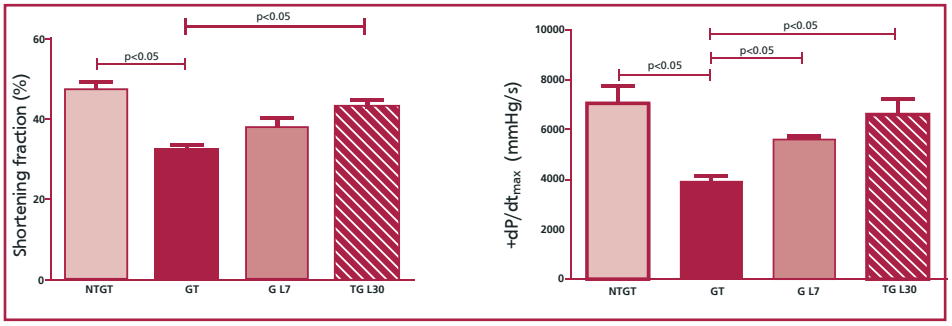

Fig. 1. Shortening fraction (panel A) and +dP/dt$_{max}$ (panel B) changes in the four study groups. Losartan treatment reverses systolic dysfunction observed in TG animals.

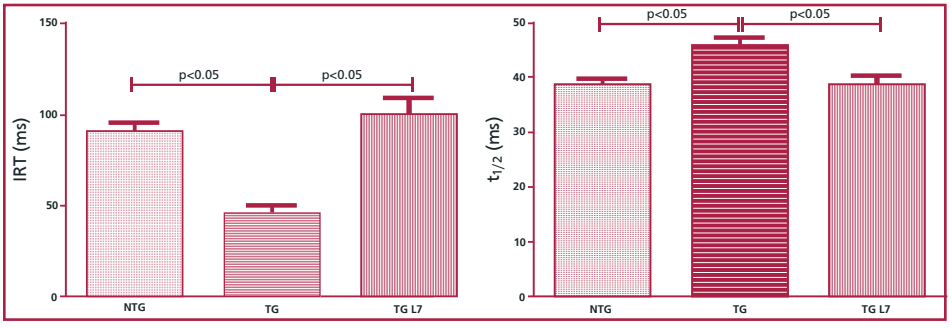

Fig. 2. Isovolumic relaxation time and t$_{½}$ changes in the four study groups. Losartan treatment for 7 or 30 days reverses abnormal isovolumic relaxation.

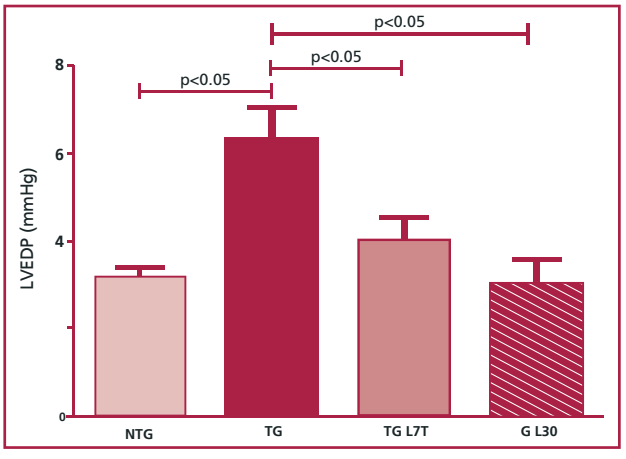

Fig. 3. Left ventricular end-diastolic pressure (LVEDP) changes show a significant increase in TG animals which is completely reversed by losartan

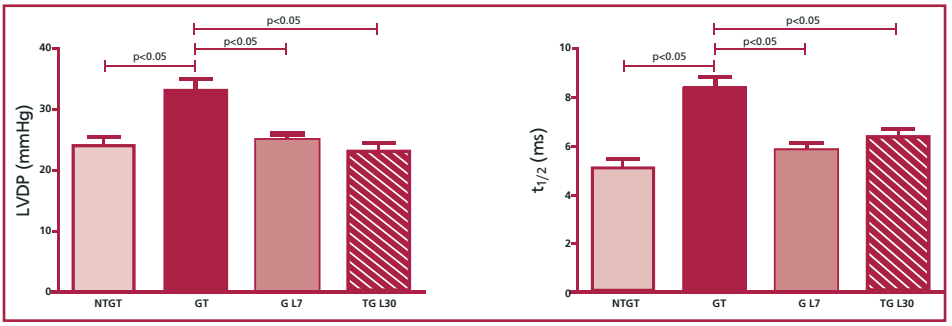

Fig. 4. Left ventricular developed pressure (LVDP) and $t_{1/2}$ in in vitro heart experiments. Hearts of TG animals present a lower contractile state and prolonged ventricular relaxation. These changes are reversed by losartan treatment for 7 days.

agree with those of Rivard K et al. (12) who showed that AT1R overexpression, with normal Ang II plasma levels, has a deleterious effect on contractility. Furthermore, our work extends this concept by showing that AT1R overexpression in mice impairs relaxation and increases ventricular diastolic pressures. This is interesting, because in models with chronic stimulation of AT1R, diastolic function in general and ventricular relaxation in particular have not been studied extensively. In that sense, some authors (18) have demonstrated that AT1R blockade prevents the diastolic dysfunction that accompanies heart failure in a model of arterial hypertension. Specifically, AT1R blockade decreased collagen synthesis, reducing ventricular stiffness with no significant changes in blood pressure.

In our study, TG mice showed diastolic dysfunction characterized by both in vivo and in vitro prolonged ventricular relaxation and increased ventricular filling pressures (LVEDP). The latter occurs without changes in left ventricular diameter, revealing increased ventricular stiffness. All these diastolic alterations were reversed with losartan treatment. Of note, AT1R blockade for 30 days reduced ventricular hypertrophy, a fact that could explain ventricular function improvement. Therefore, to discriminate whether the benefit on ventricular function was due to hypertrophy regression, we performed a group with losartan treatment for 7 days. The results clearly showed that even in the presence of ventricular hypertrophy, AT1R blockade improves systolic and diastolic function.

The mechanism by which AT1R receptor blockade improves ventricular systolic and diastolic function, even in the presence of LVH is unknown. Several intracellular proteins are involved in regulating the contractile state under these conditions. In this regard, it is known that protein kinase C (PKC) is involved in the mechanism by which Ang II exerts its inotropic effect. Thus, in isolated hearts, Ang II inotropic effect was eliminated after administration of PKC inhibitors. (19, 20) In addition, Palomeque et al (21) demonstrated that Ang II promotes a series of intracellular signals that involve PKC, tyrosine kinases and p38 MAPK activation, leading to decreased myofilament responsiveness to Ca2+ and reduced myocardial contractility.

Finally, altered ventricular relaxation as well as inotropic changes, might be linked to reduced phospholamban expression and/or phosphorylation, altering Ca2+ reuptake by SERCA 2 and prolonging relaxation. In this respect, it has been shown (20) that dual blockade with ACE inhibitors and AT1 blockers improves diastolic function in a hypertensive rat model overexpressing renin. The chronic inhibition of Ang II synthesis combined with blockade of Ang II effect on the AT-1 receptor prevented the development of LVH and the functional abnormalities of left ventricular relaxation. These findings were associated with decreased phospholamban/SERCA2 ratio.

CONCLUSIONS

Cardiac overexpression of AT1R induces ventricular systolic and diastolic dysfunction, which is completely reversed by AT1 receptor blockade. This beneficial effect is independent of left ventricular mass changes. Consequently, more studies are needed to describe the mechanism by which these drugs improve ventricular function directly, given the important role of RAS in the pathophysiological mechanism of hypertrophy and heart failure.

RESUMEN

Cambios en la función ventricular sistólica y diastólica en un modelo de sobrexpresión cardíaca de receptor AT-1 de angiotensina

La angiotensina II (Ang II) está involucrada en diferentes procesos fisiopatológicos, particularmente actuando sobre los receptores AT-1 de Ang II (AT1R). El objetivo de este trabajo fue evaluar la función ventricular sistólica y diastólica in vivo e in vitro en ratones con sobre-expresión cardíaca específica del receptor AT-1 de Ang II (AT1R). Un segundo objetivo, fue determinar si el bloqueo agudo y crónico del AT1R revierte los cambios en la función ventricular.

Se estudiaron ratones que fueron divididos en cuatro grupos experimentales. El primer grupo incluyó animales no transgénicos (NTG, n=10), el segundo grupo ratones transgénicos (TG, n=7) que sobre-expresan sólo a nivel cardíaco el AT1R y el tercer y cuarto grupo animales TG tratados con Losartan (L) durante 7 (TG L7, n=9) y 30 días (TG L30, n=7), respectivamente. Los ratones TG presentaron hipertrofia ventricular izquierda (HVI). El tratamiento con L por 7 días no revirtió la HVI, lo que si sucede cuando se extiende por 30 días. La animales TG presentan una disminución significativa de la fracción de acortamiento, desde un valor de

47.1±2.3 hasta 32.3±1.3% ($p<0.05$) y de la $+dP/dt_{máx}$ que se reduce desde un valor de 7073±674 hasta 3897.5±209.7 mmHg/seg ($p<0.05$). El tratamiento con losartan por 7 días y 30 días revierte esta disfunción sistólica

El tiempo de relajación isovolúmica y el t1/2 fueron de 24.1±1.3 y 5.1±0.5 mseg, respectivamente en los NTG. Estos índices se incrementaron a 33.1±2.2 y 8.4±0.4 mseg, respectivamente en los ratones TG ($p<0.05$). Esta alteración de la función diastólica fue revertida completamente con el tratamiento con losartan por 7 y 30 dias. El análisis de la función ventricular in vitro con control de variables, corroboró los hallazgos realizados in vivo.

La sobreexpresión cardiaca de los AT1R induce una disfunción ventricular sistólica y diastólica que es revertida completamente por el bloqueo del AT1R. Este efecto beneficioso es independiente de modificaciones en la masa ventricular izquierda.

Palabras clave > Angiotensina II, función ventricular, receptor AT-1

Conflicts of interest
None declared.

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