A role for cardiotephin-1 in myocardial remodeling induced by aldosterone

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Cardiotrophin (CT)-1 is a member of the IL-6 superfamily that exerts its cellular effects by interacting with the heterodimer constituted by the glycoprotein 130 and leukemia inhibitory factor receptor-β (21). Our group (13, 14) has already characterized CT-1-induced cardiomyocyte survival and hypertrophy. Moreover, it has been shown that CT-1 directly stimulates cardiac fibroblast proliferation and collagen type I synthesis (5, 31), suggesting a role for the cytokine in the development of myocardial fibrosis.

Our group (9, 10) has recently shown that Aldo induced CT-1 expression in both cardiac and vascular cells in vitro. Moreover, Aldo-induced CT-1 upregulation seems to play a role in the ability of the mineralocorticoid to produce cardiomyocyte growth and, as a result, LVH (10). Furthermore, elevated plasma concentrations of CT-1 have been reported in HF patients (12), and a significant association has been found between abnormally high CT-1 and abnormally high Aldo in these patients (12), suggesting that the mineralocorticoid pathways might be involved in CT-1 overproduction in HF.

Therefore, the hypothesis emerges that CT-1 could be a key factor involved in the cardiovascular remodeling induced by Aldo associated with cardiac hypertrophy and fibrosis, facilitating cardiovascular dysfunction. The present study was designed to examine the role of CT-1 in myocardial remodeling induced by Aldo in two animal models: 1) rats treated with Aldo-salt and 2) wild-type (WT) and CT-1-null mice infused with Aldo under a normal Na+ diet.

METHODS

Animals. This investigation was carried out with governmental approval (license no. B 54-547-20) and was performed in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Pub. No. 82-23, Revised 1996). Twelve-week-old male Wistar rats weighing 250 g (Harlan Ibérica, Barcelona, Spain) were randomly divided into four groups. In the control group (n = 13), rats received subcutaneous vehicle (sunflower oil) for 3 wk. In the Aldo-salt group (n = 17), rats received subcutaneous Aldo dissolved in sunflower oil (1 mg.kg−1·day−1) and 1% NaCl as drinking water for 3 wk. In the Aldo-salt + spironolactone (Spiro) group (n = 8), rats received subcutaneous Aldo (1 mg.kg−1·day−1) and Spiro (200 mg.kg−1·day−1) and 1% NaCl as drinking water. Finally, in the Spiro group (n = 5), rats received subcutaneous Spiro (200 mg.kg−1·day−1). The model and dosage of Aldo were chosen from pilot studies in which the treatment increased blood pressure and induced LVH. The plasma Aldo concentration was analyzed by a specific quantitative sandwich enzyme immunoassay (Cayman Chemical, Cayman, MI).

Adult CT-1-null mice backcrossed into a C57BL6 background were obtained from Dr. Pennica (Genentech). The experimental groups were always composed of littermate animals. Aldo-treated WT and CT-1-null mice were implanted with osmotic mini-pumps (model 2004, Alzet, 1

Address for reprint requests and other correspondence: N. López-Andrés, INSERM, U961, Faculty of Medicine, Vandoeuvre-lès-Nancy 54505, France (e-mail: natalia.lopez-andres@nancy.insERM.fr).

López-Andrés N, Martin-Fernandez B, Rossignol P, Zannad F, Lahera V, Fortuno MA, Cachofeiro V, Díez J. A role for cardiotephin-1 in myocardial remodeling induced by aldosterone. Am J Physiol Heart Circ Physiol 301: H2372–H2382, 2011.—Hyperaldosteronism is associated with left ventricular (LV) hypertrophy (LVH) and fibrosis. Cardiotrophin (CT)-1 is a cytokine that induces myocardial remodeling. We investigated whether CT-1 mediates Aldo (Aldo)-induced myocardial remodeling in two experimental models. Wistar rats were treated with Aldo-salt (1 mg.kg−1·day−1) with or without spironolactone (200 mg.kg−1·day−1) for 3 wk. Wild-type (WT) and CT-1-null mice were infused with Aldo (1 mg.kg−1·day−1) for 3 wk. Hemodynamic parameters were analyzed. LVH, fibrosis, inflammation, and CT-1 expression were evaluated in both experimental models by histopathological analysis, RT-PCR, Western blot analysis, and ELISA. Hypertensive Aldo-treated rats exhibited increased LV end-diastolic pressure and −dP/dt compared with controls. The cardiac index, LV cross-sectional area and wall thickness, cardiomyocyte size, collagen deposition, and inflammation were increased in Aldo-salt-treated rats. Myocardial expression of molecular markers assessing LVH and fibrosis as well as CT-1 levels were also augmented by Aldo-salt. Spironolactone treatment reversed all the above effects. CT-1 correlated positively with hemodynamic, histological, and molecular parameters showing myocardial remodeling. In WT and CT-1-null mice, Aldo infusion did not modify blood pressure. Whereas Aldo treatment induced LVH, fibrosis, and inflammation in WT mice, the mineralocorticoid did not provoke cardiac remodeling in CT-1-null mice. In conclusion, in experimental hyperaldosteronism, the increase in CT-1 expression was associated with parameters showing LVH and fibrosis. CT-1-null mice were resistant to Aldo-induced LVH and fibrosis. These data suggest a key role for CT-1 in cardiac remodeling induced by Aldo independent of changes in blood pressure levels. Fibrosis; left ventricular hypertrophy; inflammation

ALDOSTERONE (Aldo) is a mineralocorticoid hormone that has been associated with the development of cardiovascular remodeling, fibrosis, and injury (1, 2, 4, 27, 35). Myocardial fibrosis and left ventricular (LV) hypertrophy (LVH) are hallmarks of most cardiac pathologies and facilitate the development of heart failure (HF) (25, 29). Accumulating clinical evidence shows that an excess of Aldo in patients with primary hyperaldosteronism is associated with LVH and alterations in myocardial texture (23, 24). Moreover, blockade of the mineralocorticoid receptor has been shown to improve outcome, LVH, and fibrosis in patients with HF (22, 36, 37).
Cardiomyocyte width, \( m \) 31.5
Cardiomyocyte length, \( \mu m \) 97.1
Cardiomyocyte width, \( \mu m \) 2.3
Atrial natriuretic peptide
mRNA, AU 3.9
c-fos mRNA, AU 0.10
C-fos protein, AU 2.9
C-Myc protein, AU 2.6

Values are means ± SE; \( n \), no. of animals. LVCSA, LV cross-sectional area; LVWT, LV wall thickness; AU, arbitrary units. \(* P < 0.01 \) vs. control; † \( P < 0.01 \) vs. control; ‡ \( P < 0.05 \) vs. control.
treated rats compared with control rats. Moreover, at death, plasma Aldo was significantly increased in Aldo-salt-treated animals compared with control animals (700 ± 144 vs. 275 ± 42 pg/ml, *P* < 0.01).

Average body weight (BW) and heart weight (HW) as well as blood pressure and cardiac function for control, Aldo-salt-, and Aldo-salt + Spiro-infused rats are shown in Table 1. HW and the HW-to-BW ratio (HW/BW) of Aldo-treated rats were significantly increased (*P* < 0.01) relative to control rats. Moreover, Aldo-salt induced an increase (*P* < 0.01) in SBP, DBP, and MAP. Values of LVSP were higher (*P* < 0.01) in the Aldo-salt-treated group and +dP/dt was similar in both groups, indicating a preserved systolic function after Aldo-salt treatment. However, LVEDP and −dP/dt were augmented (*P* < 0.01) in rats treated with Aldo-salt, demonstrating an altered diastolic function. Moreover, heart rate was decreased in Aldo-salt-treated rats compared with control rats. Cardiac hypertrophy and hypertension as well as diastolic dysfunction were fully prevented by Spiro treatment. All the above parameters were unaffected by Spiro alone (data not shown).

**Effects of Aldo-salt treatment in myocardial remodeling in rats.** Cardiac histological and molecular analyses are shown in Table 2. Aldo-salt-treated rats exhibited increased LVCSA (16%, *P* < 0.01) and LVWT (14%, *P* < 0.01) compared with control rats. Interstitial fibrosis was increased in Aldo-salt-treated animals. Spiro blocked Aldo-salt-induced interstitial fibrosis. Aldo-salt-treated rats presented augmented perivascular fibrosis compared with control and Aldo-salt + Spiro rats. The expression of extracellular matrix components was quantified by RT-PCR and Western blot analysis. 18S rRNA gene expression or β-actin levels were used as loading controls in RT-PCR and Western blot analysis, respectively. Aldo-salt-treated rats presented increased α1-procollagen, collagen type I-to-III ratio, and matrix metalloproteinase (MMP)-13-to-tissue inhibitor of metalloproteinase (TIMP)-1 ratio. *P* < 0.01 vs. control.

Fig. 1. Morphology and composition of the myocardium from control, aldosterone (Aldo)-salt, and Aldo-salt + spironolactone (Spiro)-treated rats. A: representative photomicrographs of myocardial Masson’s trichrome-stained sections. B: interstitial myocardial fibrosis was evaluated in Sirius red-stained sections. After 3 wk of treatment, interstitial collagen deposition was increased in Aldo-salt-treated animals. Spiro blocked Aldo-salt-induced myocardial fibrosis. C: perivascular myocardial fibrosis was quantified in sections stained with Sirius red. Aldo-salt-treated rats presented augmented perivascular fibrosis compared with control and Aldo-salt + Spiro rats. D: the expression of extracellular matrix components was quantified by RT-PCR and Western blot analysis. 18S rRNA gene expression or β-actin levels were used as loading controls in RT-PCR and Western blot analysis, respectively. Aldo-salt-treated rats presented increased α1-procollagen, collagen type I-to-III ratio, and matrix metalloproteinase (MMP)-13-to-tissue inhibitor of metalloproteinase (TIMP)-1 ratio. *P* < 0.01 vs. control.
control rats (representative photographs of heart sections are shown in Fig. 1A). At the cellular level, Aldo-salt but not vehicle treatment increased cardiomyocyte length (15%, $P < 0.01$) and cardiomyocyte width (28%, $P < 0.01$). Finally, at the molecular level, Aldo-salt treatment enhanced mRNA expression of atrial natriuretic peptide (2.8-fold, $P < 0.01$) and c-fos (11-fold, $P < 0.01$) and protein expression of c-Fos (4-fold, $P = 0.01$) and c-Myc (5.5-fold, $P < 0.01$). Spiro treatment completely prevented myocardial hypertrophy at both the histological and molecular levels. All the above parameters were unaffected by Spiro alone (data not shown).

The effect of Aldo-salt treatment on collagen content in the myocardium is shown in Fig. 1. Aldo-salt-infused rats presented a fourfold increase ($P < 0.01$) in cardiac interstitial collagen (Fig. 1B) and a threefold increase ($P < 0.01$) in perivascular collagen (Fig. 1C). Animals treated with Aldo-salt + Spiro presented similar interstitial and perivascular collagen as control animals. As shown in Fig. 1D, molecular analyses were also performed in cardiac tissue to study the variations in the expression of the extracellular matrix components, mainly collagen and collagenase activity. Aldo-salt-infused rats presented a higher expression of α1-procollagen mRNA (4-fold, $P < 0.01$) as well as the ratio between collagen types I and III (5-fold, $P < 0.01$) and the ratio between matrix metalloproteinase-13 and tissue inhibitor of metalloproteinase-1 expression (2.3-fold, $P < 0.01$). Importantly, Spiro treatment abolished Aldo-salt-induced myocardial fibrosis. Spiro alone did not modify collagen deposition (data not shown).

**Effects of Aldo-salt treatment in myocardial inflammation in rats.** As shown in Fig. 2A, Aldo-salt infusion led to focal inflammatory lesions. The number of focal inflammatory clusters was 8 clusters/section (2 mm$^2$) in the LV, whereas no focal inflammatory lesions were observed in the control, Aldo + Spiro, and Spiro groups. Complementary, the number of CD3-positive cells, a T lymphocyte marker, was higher in Aldo-salt-treated rats compared with control rats (7-fold, $P < 0.01$). Spiro treatment abolished the Aldo-salt-induced increase in infiltrating inflammatory cells.

We also examined the effect of Aldo-salt on the expression of plasma inflammatory markers. Aldo-salt-treated rats presented enhanced C-reactive protein (2.6-fold, $P < 0.01$) and TNF-α (11.6-fold, $P < 0.01$) levels (Fig. 2B) compared with control rats. Spiro treatment abolished the Aldo-salt-induced increase in inflammatory markers.

**Effects of Aldo-salt treatment on CT-1 expression in rats.** To localize CT-1 in the rat myocardium, paraffin-embedded sections were labeled with CT-1 antibody. High-intensity labeling was present in cardiomyocytes and fibroblasts (Fig. 3A). Although staining was generally more intense in cardiomyocytes,
both vascular smooth muscle cells and endothelial cells from blood vessels throughout the cardiac tissue were also positive for CT-1 staining (Fig. 3B). A negative control (a section from a CT-1-null mouse) is shown in Fig. 3C. All these results were confirmed in vitro by RT-PCR in cultured cells (not shown).

Myocardial expression of CT-1 was higher both at the protein (Fig. 3D) and mRNA (Fig. 3E) levels (2.2- and 7.3-fold, respectively, \( P < 0.01 \)) in Aldo-salt-treated animals. Whereas the increase in CT-1 protein expression was completely prevented by Spiro, the mineralocorticoid receptor antagonist did not block the rise in CT-1 mRNA expression. Spiro alone did not modify myocardial CT-1 levels (data not shown).

**Analysis of the associations in rats.** Direct correlations were found among myocardial CT-1 protein expression and hemodynamic parameters, as shown in Table 3. Myocardial CT-1 expression was directly associated with SBP, DBP, MAP, LVSP, and LVEDP.

Moreover, as shown in Table 4, there were direct correlations among myocardial CT-1 protein expression and parameters assessing LVH (i.e., LVCSA and cardiomyocyte dimensions) and fibrosis (i.e., collagen type I-to-III ratio and interstitial fibrosis) in all treated rats. Multiple linear regression analysis demonstrated that, when adjusted for confounding factors (i.e., blood pressure levels), the direct associations between CT-1 and cardiomyocyte length and width, \( \alpha_1 \)-procollagen, collagen type I-to-III ratio, and interstitial collagen remained significant.

**Effects of Aldo without Na\(^+\) excess in general and haemodynamic parameters in WT and CT-1-null mice.** As shown in Table 5, BW was similar in WT and CT-1-null mice treated with vehicle or Aldo. Of note, HW was higher (\( P < 0.05 \)) in both WT and CT-1-null mice treated with Aldo than in their respective control group. However, only WT mice infused with Aldo presented an increase (\( P < 0.05 \)) in HW/BW, indicating cardiac hypertrophy. Aldo failed to increase blood pressure levels in WT and CT-1-null mice, and the four experimental groups of animals had similar values of SBP, DBP, MAP, and HR. HW and HW/BW were slightly increased (6% and 5%, respectively, \( P < 0.05 \)) in control CT-1-null mice compared with control WT mice.

**Table 3. Associations found between myocardial CT-1 expression and hemodynamic parameters**

<table>
<thead>
<tr>
<th>CT-1 Protein</th>
<th>( r )</th>
<th>( P ) value</th>
</tr>
</thead>
<tbody>
<tr>
<td>SBP, mmHg</td>
<td>0.685</td>
<td>0.001</td>
</tr>
<tr>
<td>DBP, mmHg</td>
<td>0.646</td>
<td>0.007</td>
</tr>
<tr>
<td>MAP, mmHg</td>
<td>0.623</td>
<td>0.003</td>
</tr>
<tr>
<td>LVEDP, mmHg</td>
<td>0.549</td>
<td>0.040</td>
</tr>
<tr>
<td>LVSP, mmHg</td>
<td>0.733</td>
<td>0.002</td>
</tr>
</tbody>
</table>

CT-1, cardiotrophin-1.
Effects of Aldo without Na⁺ excess in myocardial remodeling in WT and CT-1-null mice. Cardiac morphometric and molecular analyses are shown in Table 6. WT mice infused with Aldo presented increased LVCSA (11%, \( P < 0.05 \)) and augmented LVWT (13%, \( P < 0.05 \)) that were accompanied by an increase in cardiomyocyte width (6%, \( P < 0.05 \)). On the other hand, Aldo failed to induce histological modifications in hearts from mice lacking CT-1 (representative photographs are shown in Fig. 3A). LVCSA and LVWT were slightly increased (9% and 6%, respectively, \( P < 0.05 \)) in control CT-1-null mice compared with control WT mice. At the molecular level, Aldo treatment increased myocardial expression of c-fos (2.4-fold, \( P < 0.05 \)) and c-myc (2.5-fold, \( P < 0.05 \)) in WT mice, whereas in CT-1-null mice, the increases were significantly lower (c-fos: 50%, \( P < 0.05 \), and c-myc: 88%, \( P < 0.05 \)). c-fos and c-myc expressions were decreased (8-fold, \( P < 0.01 \), and 40%, \( P < 0.05 \), respectively) in control CT-1-null mice compared with control WT mice.

The effect of Aldo treatment on collagen content in the myocardium from WT and CT-1-null mice is shown in Fig. 4. Aldo-infused WT mice presented a 250% increase (\( P < 0.01 \)) in cardiac interstitial collagen, whereas Aldo-infused CT-1-null mice only presented a 70% increase (\( P < 0.05 \); Fig. 4B). Aldo induced a 166% increase (\( P < 0.01 \)) in perivascular collagen in WT mice, whereas the mineralocorticoid failed to augment perivascular collagen in CT-1-null mice (Fig. 4C). As shown in Fig. 4D, Aldo-infused WT mice exhibited a higher expression of \( \alpha_1 \)-procollagen mRNA (2.5-fold, \( P < 0.01 \)) as well as the ratio between collagen types I and III (1.7-fold, \( P < 0.05 \)). However, in mice lacking CT-1, Aldo infusion increased only \( \alpha_1 \)-procollagen mRNA (80%, \( P < 0.05 \)), without modifying the the collagen type I-to-III ratio. The effects of Aldo on interstitial fibrosis and \( \alpha_1 \)-procollagen mRNA expression were significantly different in WT mice compared with CT-1-null mice (\( P < 0.05 \)).

Effects of Aldo without Na⁺ excess in myocardial inflammation in WT and CT-1-null mice. As shown in Fig. 5A, Aldo treatment caused the appearance of more focal inflammatory lesions in WT mice than in CT-1-null mice. The number of focal inflammatory clusters in WT mice was 3 clusters/section (0.75 mm²) in the LV, whereas in CT-1-null mice the number was 2 clusters/section (0.4 mm²). No focal inflammatory lesions were observed in the control groups of WT and CT-1-null mice. Complementary, CD3 immunostaining revealed that in response to Aldo, WT mice accumulated more CD3-positive cells (345%) than CT-1-null mice (265%). The effects of Aldo on the number of focal inflammatory lesions and CD45 immunostaining were significantly different in WT mice compared with CT-1-null mice (\( P < 0.05 \)).

Both WT and CT-1-null mice treated with Aldo presented enhanced plasma C-reactive protein (90% and 88%, \( P < 0.01 \), respectively). However, plasma TNF-\( \alpha \) expression was only augmented by Aldo in WT mice (75%, \( P < 0.01 \); Fig. 5B).

Effects of Aldo without Na⁺ excess on CT-1 expression in WT mice. Myocardial expression of CT-1 were higher (2.3-fold, \( P < 0.01 \)) in normotensive Aldo-treated WT mice compared with control mice, as shown in Fig. 6.

**DISCUSSION**

The purpose of this study was to investigate the role of CT-1 in cardiovascular remodeling induced by Aldo in rodents.

### Table 4. Associations found between myocardial CT-1 expression and parameters assessing myocardial remodeling

<table>
<thead>
<tr>
<th>Parameter</th>
<th>( r )</th>
<th>( P ) value</th>
</tr>
</thead>
<tbody>
<tr>
<td>LVCSA, mm²</td>
<td>0.549</td>
<td>0.034</td>
</tr>
<tr>
<td>LVWT, mm</td>
<td>0.497</td>
<td>0.045</td>
</tr>
<tr>
<td>Cardiomyocyte length, μm</td>
<td>0.539</td>
<td>0.026</td>
</tr>
<tr>
<td>Cardiomyocyte width, μm</td>
<td>0.713</td>
<td>0.001</td>
</tr>
<tr>
<td>c-Fos protein</td>
<td>0.645</td>
<td>0.004</td>
</tr>
<tr>
<td>c-Myc protein</td>
<td>0.621</td>
<td>0.006</td>
</tr>
<tr>
<td>( \alpha_1 )-Procollagen mRNA</td>
<td>0.893</td>
<td>0.001</td>
</tr>
<tr>
<td>Collagen type I/collagen type III, AU</td>
<td>0.542</td>
<td>0.036</td>
</tr>
<tr>
<td>MMP-13/TIMP-1, AU</td>
<td>0.522</td>
<td>0.026</td>
</tr>
<tr>
<td>Interstitial collagen, %</td>
<td>0.624</td>
<td>0.003</td>
</tr>
<tr>
<td>Perivascular collagen, %</td>
<td>0.568</td>
<td>0.036</td>
</tr>
</tbody>
</table>

### Table 5. General and hemodynamic parameters in WT and CT-1-null mice infused with Aldo

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>Aldo</th>
<th>Control</th>
<th>Aldo</th>
</tr>
</thead>
<tbody>
<tr>
<td>( n )</td>
<td>8</td>
<td>8</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>BW, g</td>
<td>28.7 ± 0.6</td>
<td>28.1 ± 0.5</td>
<td>28.7 ± 0.4</td>
<td>29.7 ± 0.5</td>
</tr>
<tr>
<td>HW, g</td>
<td>0.157 ± 0.004</td>
<td>0.168 ± 0.004*</td>
<td>0.166 ± 0.006</td>
<td>0.172 ± 0.007*</td>
</tr>
<tr>
<td>HW/BW, mg/g</td>
<td>5.49 ± 0.2</td>
<td>5.99 ± 0.1*</td>
<td>5.79 ± 0.2</td>
<td>5.82 ± 0.3</td>
</tr>
<tr>
<td>SBP, mmHg</td>
<td>122 ± 2</td>
<td>123 ± 4</td>
<td>114 ± 2</td>
<td>116 ± 3</td>
</tr>
<tr>
<td>DBP, mmHg</td>
<td>101 ± 1</td>
<td>102 ± 4</td>
<td>101 ± 2</td>
<td>92 ± 3</td>
</tr>
<tr>
<td>MAP, mmHg</td>
<td>112 ± 2</td>
<td>112 ± 4</td>
<td>105 ± 2</td>
<td>104 ± 3</td>
</tr>
<tr>
<td>HR, beats/min</td>
<td>627 ± 12</td>
<td>630 ± 20</td>
<td>606 ± 23</td>
<td>606 ± 22</td>
</tr>
</tbody>
</table>

Values are means ± SE; \( n \), no. of animals. WT, wild type. *\( P < 0.05 \) vs. control.
CARDIOTROPHIN-1 MEDIATES ALDOSTERONE-INDUCED CARDIAC REMODELING

A

WT

Control

Aldo

CT-1-null

B

WT

Control

Aldo

CT-1-null

C

WT

Control

Aldo

CT-1-null

D

Ratio of collagen type I and III

Control

Aldo

WT

CT-1-null

Collagen type I

Collagen type III (AU)

Control

Aldo

WT

CT-1-null
Here, we demonstrate, for the first time, that Aldo induced CT-1 expression in vivo, independent from blood pressure levels. Moreover, the increased CT-1 was associated with hemodynamic parameters and with parameters showing LVH and fibrosis. The data obtained in CT-1-null mice clearly demonstrate that CT-1 is a key mediator in Aldo-induced harmful myocardial effects.

Numerous studies have documented that increased Aldo, in addition to excess dietary salt and the removal of one kidney, elevates blood pressure and induces LVH, fibrosis, inflammatory responses, and dysfunction (3, 15, 16, 18, 20, 28) and that the effects of Aldo are crucially dependent on the salt status of the rat (2). On the other hand, it has been described that excess Aldo under a normal salt diet, with minor blood pressure elevation, plays a pivotal role in cardiac remodeling and inflammation (34). Therefore, in the present study, two different models were used: rats treated with Aldo-salt, which presented elevated arterial pressure, and mice treated with Aldo not subjected to Na\(^+\) loading, which presented normal blood pressure levels. In normal nonuninephrectomized rats, Aldo-salt infusion increased blood pressure and also induced diastolic dysfunction accompanied by LVH, fibrosis, and inflammation, as previously reported (3, 15, 16, 18, 20, 28). The mineralocorticoid receptor blocker Spiro inhibited Aldo-induced high blood pressure and myocardial remodeling. These data are in accordance with results showing that in experimental models, blockade of the mineralocorticoid receptor improved not only LVH and fibrosis but also diastolic dysfunction (6). Furthermore, Spiro treatment improved myocardial inflammation, zinc dyshomeostasis, and oxidative stress (28, 30, 34). Finally, it is important to point out that mineralocorticoid receptor blockade improves outcome and myocardial remodeling in patients with HF (22, 36, 37). In WT mice subjected to the same dose of Aldo, the mineralocorticoid failed to induce blood pressure modifications, but it induced LVH, fibrosis, and inflammatory lesions. The fact that Aldo did not cause hypertension but induced LVH, fibrosis, and inflammation in mice suggests that it has direct cardiac effects independent of changes in blood pressure. Our data differed from previous findings in regard to the slight increase in blood pressure levels induced by Aldo without Na\(^+\) excess in rats (34). The difference between our present observations and previous findings could be related to the fact that the mice were not uninephrectomized and the fact that C57BL6 mice seem to be resistant to hypertensive stimuli (7, 26, 32, 33).

Molecular mechanisms underlying Aldo-induced LVH and fibrosis have to be delineated. Previous studies have suggested the involvement of ANG II (8), oxidative stress (28, 34), magnesium (26), apoptosis signal-regulating kinase-1 (17), transforming growth factor-β (15), or connective tissue growth factor (15). We (9, 10) have previously published that Aldo induced the expression of CT-1, a profibrotic and hypertrophic cytokine, in both adult cardiomyocytes and vascular cells, suggesting a role for the cytokine in Aldo-induced cardiomyocyte hypertrophy and vascular alterations. In the present study, we found that Aldo-salt hypertensive rats, as well as normotensive Aldo-treated mice, exhibited increased myocardial CT-1, and the cytokine was associated with parameters showing myocardial remodeling independent of blood pressure levels. Moreover, CT-1 was associated with LV pressure, suggesting that the cytokine is produced as a response to mechanical stress presented in the myocardial wall. The fact that CT-1 is expressed in cardiac myocytes, fibroblasts, and vascular cells reinforces the associations found between the cytokine and Aldo-induced effects on hemodynamics, hypertrophy, and interstitial and perivascular fibrosis. Related to this, previous studies (11, 14, 19) have reported CT-1 elevations in both experimental models and patients with hypertension. Moreover, in HF patients, high Aldo levels were associated with high CT-1 levels (12). Therefore, the possible role of hemodynamic factors in CT-1 induction remains uncertain. However, CT-1 was also increased in the myocardium from normotensive WT mice infused with Aldo, indicating that CT-1 induction by Aldo is independent of hemodynamic factors. Finally, we demonstrated that CT-1 is a necessary factor for the accumulation of fibrous tissue and the development of LVH induced by Aldo. Indeed, Aldo failed to induce LVH and fibrosis in mice lacking CT-1, suggesting that the absence of the cytokine blocked the Aldo-induced effects on cardiac remodeling. Moreover, in the absence of CT-1, Aldo proinflammatory effects were also diminished. Thus, our present findings provide a new molecular mechanism underlying the cardiac remodeling and injury caused by Aldo in vivo and independent of hemodynamic changes.

In conclusion, our present findings provide the first evidence that CT-1, independent of hypertension, significantly participates in Aldo-induced LVH and fibrosis. Therefore, we suggest that CT-1 could be a new biotarget to reduce the cardiac remodeling induced by Aldo in cardiovascular diseases.

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Fig. 4. Morphology and composition of the myocardium from wild-type (WT) and CT-1-null mice infused with Aldo without Na\(^+\) excess. A: representative photomicrographs of myocardial Masson’s trichrome-stained sections from WT and CT-1-null mice treated with vehicle or Aldo. B: interstitial myocardial fibrosis was evaluated in Sirius red-stained sections. After 3 wk of treatment, Aldo increased interstitial collagen deposition in WT mice, whereas the increase was significantly lower in CT-1-null mice. The effect of Aldo on interstitial fibrosis was significantly higher in WT mice compared with CT-1-null mice. *P < 0.05 vs. control (same strain); $P < 0.05 vs. WT mice (same treatment).
Fig. 5. Inflammation in the myocardium from WT and CT-1-null mice infused with Aldo without Na⁺ excess. A: representative photomicrographs of Aldo-treated myocardial Masson’s trichrome-stained sections from WT and CT-1-null mice showing focal inflammatory lesions. Aldo-treated WT mice presented more and larger focal inflammatory lesions than Aldo-treated CT-1-null mice. B: representative photomicrographs of Aldo-treated myocardial CD3-stained sections from WT and CT-1-null mice demonstrating infiltrating inflammatory cells. Aldo-treated WT mice presented higher immunostaining than Aldo-treated CT-1-null mice. C: CRP plasma levels were enhanced in both WT and CT-1-null mice treated with Aldo, whereas TNF-α plasma levels were enhanced only in Aldo-treated WT mice. ∗P < 0.05 vs. control (same strain); $P < 0.05$ vs. WT mice (same treatment).
Fig. 6. CT-1 expression in myocardial tissue from control mice treated with Aldo without Na+ excess. CT-1 expression was quantified by ELISA. Aldo treatment enhanced myocardial CT-1 expression in WT mice compared with control mice. *P < 0.01 vs. control.

DISCLOSURES
No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS
Author contributions: N.L.-A., P.R., F.Z., V.L., M.A.F., V.C., and J.D. conceived and designed the research; N.L.-A. and B.M.-F. performed experiments; N.L.-A. and B.M.-F. analyzed data; N.L.-A., B.M.-F., V.I., M.A.F., V.C., and J.D. interpreted results of experiments; N.L.-A. and B.M.-F. wrote the manuscript; N.L.-A., B.M.-F., P.R., F.Z., V.L., M.A.F., V.C., and J.D. approved final version of manuscript.

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