Augmented Cardiac Cardiotrophin-1 in Experimental Congestive Heart Failure

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Background—Cardiotrophin-1 (CT-1) is a potent hypertrophic factor discovered by coupling expression cloning in a mouse embryonic stem cell–based model of cardiogenesis.

Methods and Results—The present study was designed to investigate the potential activation of atrial and ventricular CT-1 expression in pacing-induced experimental congestive heart failure (CHF) and its relationship to left ventricular hypertrophy by the method of Northern blot analysis and immunohistochemistry. We used a canine model of pacing-induced experimental CHF based on hemodynamic and neurohumoral characteristics that closely mimic human dilated cardiomyopathy. Northern blot analysis demonstrated that CT-1 gene expression was present in normal atrium and ventricle and was increased in CHF hearts. There was a positive correlation between ventricular CT-1 mRNA and left ventricular mass index. Immunohistochemistry revealed positive immunostaining in the atrial and ventricular cardiomyocytes from both normal and CHF hearts. CT-1 immunoreactivity was more intense in the atrium and ventricle from CHF hearts than in normal hearts.

Conclusions—The present study demonstrates that both atrium and ventricle synthesize CT-1 and that cardiac production of CT-1 is augmented in a canine model of experimental CHF. This study also demonstrates that ventricular CT-1 mRNA correlates with left ventricular hypertrophy, suggesting that CT-1 plays an important role in the structural remodeling that characterizes CHF. \(\textit{Circulation.} 2000;101:14-17.\)

Key Words: genes • immunohistochemistry • hypertrophy • heart failure

A fundamental characteristic of congestive heart failure (CHF) is the development of ventricular hypertrophy, which occurs in response to chronic volume and/or pressure overload. Although the initiating molecular pathway for myocardial hypertrophy is unknown, recent evidence suggests an important role for the interleukin-6 (IL-6)–type cytokines in association with Janus kinase–signal transducers and activators of transcription (JAK-STAT) cascade pathway.1,2

In 1995, Pennica et al3 reported that culture medium from mouse embryoid bodies exerted potent hypertrophic actions on cultured cardiomyocytes. Expression cloning revealed that this hypertrophic action on cardiomyocytes was caused by a 21.5-kD protein, cardiotrophin-1 (CT-1). CT-1–induced cardiomyocyte hypertrophy in vitro resembles the hypertrophic pattern observed in volume overload hypertrophy in human CHF.4 Subsequently, CT-1 was identified as a member of a family of IL-6–type cytokines that includes IL-6, IL-11, leukemia inhibitory factor, oncostatin M, and ciliary neurotrophic factor.5 Among these cytokines, high serum IL-6 concentration was associated with poor prognosis in patients with CHF.6 In addition, although previous investigations reported increased cardiac CT-1 in parasite-induced Chagas disease7 and in genetically hypertensive rats,8 it is unknown to date whether CT-1 is activated in CHF. Furthermore, myocardial CT-1 gene expression and its relationship to left ventricular hypertrophy in CHF remain undefined.

In the present investigation, we used a canine model of pacing-induced experimental CHF whose hemodynamic and neurohumoral characteristics closely mimic human dilated cardiomyopathy with ventricular hypertrophy.9 We defined atrial and ventricular CT-1 gene expression by Northern blot analysis and CT-1 immunoreactivity by immunohistochemistry. We also defined the relationship between CT-1 gene expression and left ventricular hypertrophy.

Methods

Experimental Canine Model of CHF

Experimental CHF was produced in 5 mongrel dogs by rapid ventricular pacing as reported previously.9 During the first 10 days, the dogs were paced at 180 bpm, and the pacing rate was increased weekly to 200, 210, 220, and 240 bpm to produce overt CHF with ventricular hypertrophy. In addition, these dogs underwent implantation of a femoral artery catheter via the left femoral artery and subcutaneously connected to a port above the upper hind limb. At baseline before pacing and after being paced at 240 bpm (day 38), hemodynamic measurement and blood sampling were performed as...
previously reported. At the end of the pacing, the dogs were killed, and the cardiac tissues were rapidly harvested. Five age-matched normal mongrel dogs served as cardiac tissue donors. This study was approved by the institutional animal care and use committee of the Mayo Clinic and conducted in accordance with the Animal Welfare Act.

**Measurement of Neurohumoral Factors**

Atrial natriuretic peptide, cGMP, plasma renin activity, and aldosterone were determined by radioimmunoassay techniques as previously reported.

**Reverse Transcription–Polymerase Chain Reaction**

Messenger RNA was isolated from canine atrium and ventricle as previously reported. First-strand cDNA was synthesized from 1 μg of canine atrial mRNA with oligo(dT) primer and Moloney murine leukemia virus reverse transcriptase. For amplification of canine CT-1 cDNA fragment, the following primers were selected: sense, 5′-AGCATGAGCCGGAGGGAA-3′; antisense, 5′-TTCCCTGGAGCAGCTGCTCAGCATAT-3′. The polymerase chain reaction products were cloned into a vector and sequenced by the ABI Prism dideoxy chain termination method (GenBank accession number AF095589).

**Northern Blot Analysis**

Northern blot analysis was performed as previously reported. To standardize loading conditions and mRNA transfer onto membranes, blots were rehybridized with a GAPDH probe.

**Immunohistochemistry**

Immunohistochemical studies were performed with the indirect immunoperoxidase method as previously described. The staining intensity of CT-1 immunoreactivity was evaluated semiquantitatively from 0 to 4 (0, absence of any staining; 1, minimal intensity; 2, mild intensity; 3, moderate intensity; and 4, maximal intensity).

**TABLE 1. Hemodynamic and Neurohumoral Data in Baseline Before Pacing and in CHF**

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>CHF</th>
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<tbody>
<tr>
<td>Mean arterial pressure, mm Hg</td>
<td>108±3</td>
<td>91±6*</td>
</tr>
<tr>
<td>Right atrial pressure, mm Hg</td>
<td>5±1</td>
<td>13±2*</td>
</tr>
<tr>
<td>Pulmonary artery pressure, mm Hg</td>
<td>18±1</td>
<td>27±2*</td>
</tr>
<tr>
<td>Pulmonary capillary wedge pressure, mm Hg</td>
<td>10±1</td>
<td>22±2*</td>
</tr>
<tr>
<td>Cardiac output, L/min</td>
<td>5.1±0.4</td>
<td>2.2±0.3*</td>
</tr>
<tr>
<td>Systemic vascular resistance, mm Hg · L–1 · min–1</td>
<td>20.7±1.9</td>
<td>40.2±5.6*</td>
</tr>
<tr>
<td>Pulmonary vascular resistance, mm Hg · L–1 · min–1</td>
<td>1.7±0.3</td>
<td>2.9±0.8</td>
</tr>
<tr>
<td>Left ventricular end-diastolic diameter, mm</td>
<td>39±1</td>
<td>47±1*</td>
</tr>
<tr>
<td>Left ventricular end-systolic diameter, mm</td>
<td>27±1</td>
<td>42±1*</td>
</tr>
<tr>
<td>Ejection fraction, %</td>
<td>53±1</td>
<td>23±2*</td>
</tr>
<tr>
<td>Atrial natriuretic peptide, pg/mL</td>
<td>34±4</td>
<td>478±108*</td>
</tr>
<tr>
<td>cGMP, pmol/mL</td>
<td>4.4±0.1</td>
<td>19.0±1.9*</td>
</tr>
<tr>
<td>Plasma renin activity, ng · mL–1 · h–1</td>
<td>0.9±0.4</td>
<td>6.7±1.9*</td>
</tr>
<tr>
<td>Aldosterone, ng/dL</td>
<td>2.7±0.2</td>
<td>31.2±12.3*</td>
</tr>
</tbody>
</table>

*P<0.05 vs baseline.

Figure 1. A, Northern blot analysis for CT-1 in the atrium and ventricle from normal and CHF hearts (GenBank accession number AF095589). GAPDH probe was used as a normalizing control in Northern blot analysis. B, Correlation between ventricular CT-1 mRNA and left ventricular mass index (y=2.961x+3.630, r=0.91, P<0.0002). AU indicates arbitrary units.

**Statistical Analysis**

Values are expressed as mean±SEM. Statistical comparisons between before and after pacing were performed by Student’s paired t test. Statistical comparisons between normal and CHF dogs were performed by Student’s unpaired t test. The correlation analysis was performed by linear regression analysis. Statistical significance was accepted for P<0.05.

**Results**

**Hemodynamic and Neurohumoral Data**

Experimental CHF was characterized by decreases in mean arterial blood pressure, cardiac output, and left ventricular ejection fraction with increases in cardiac filling pressures and systemic vascular resistance. Left ventricular systolic and diastolic diameters increased in CHF. Experimental CHF was also characterized by neurohumoral activation (Table 1). All CHF dogs had pulmonary edema, pleural effusion, and ascites.

**Northern Blot Analysis**

CT-1 mRNA was detected in atrium and ventricle, and both atrial and ventricular CT-1 mRNA increased in CHF (Figure 1). Densitometric analysis revealed that the ratio of CT-1 mRNA to GAPDH mRNA increased from 0.33±0.02 to
0.72±0.05 in atria (P<0.05) and from 0.21±0.03 to 0.45±0.04 in ventricles (P<0.05) in CHF hearts compared with normal hearts.

**Relationship Between CT-1 Gene Expression and Left Ventricular Hypertrophy**

Left ventricular mass index (left ventricular weight divided by body weight) increased from 4.2±0.2 g/kg in normal dogs to 4.9±0.3 g/kg in CHF (P<0.05). Ventricular CT-1 gene expression correlated positively with left ventricular mass index (y=2.961x+3.630, r=0.91, P<0.0002) (Figure 1).

**Immunohistochemistry**

CT-1 immunoreactivity was observed in the cytoplasm of cardiomyocytes and was distributed widely in the peripheral cytoplasm (Figure 2). The immunostaining scores were increased in both atrium and ventricle of CHF hearts compared with normal hearts (Table 2).

<table>
<thead>
<tr>
<th></th>
<th>Normal</th>
<th>CHF</th>
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<tbody>
<tr>
<td>Atrium</td>
<td>0.8±0.1</td>
<td>3.4±0.1*</td>
</tr>
<tr>
<td>Ventricle</td>
<td>0.5±0.1</td>
<td>2.6±0.2*</td>
</tr>
</tbody>
</table>

*P<0.05 vs normal.

**Discussion**

This study demonstrates for the first time that CT-1 gene expression and immunoreactivity are present in the normal canine myocardium and increased in both atrium and ventricle in experimental CHF. On the basis of the potent growth-promoting properties of CT-1 in vitro, the strong relationship in vivo between CT-1 gene expression and left ventricular hypertrophy supports a possible role of CT-1 in the hypertrophic process associated with CHF. Thus, through signaling pathways that include the signaling subunit gp130 and subsequent signaling pathways, augmented cardiac CT-1...
may play an important role in cardiac hypertrophy in dilated cardiomyopathy, which is supported by our finding in pacing-induced experimental CHF.

The present immunohistochemical staining reveals CT-1 immunoreactivity in the normal canine heart and that CT-1 immunoreactivity is augmented in both atrium and ventricle in experimental CHF, thus paralleling the increase in CT-1 mRNA. Three unsolved questions remain to be addressed in the present report. The first addresses the mechanisms of activation of CT-1 in CHF. Our model of CHF involves ventricular dilatation and neurohumoral stimulation. Although both mechanical and neurohumoral factors have been associated with ventricular hypertrophy, both should be investigated as to whether they provide a stimulus for CT-1 gene activation. The second concern is the causal role for CT-1 in vivo in CHF. Previous studies have reported ventricular hypertrophy in transgenic models that overexpress IL-6 and its receptor (IL-6R). Most importantly, an increase in both IL-6 and its receptor was necessary to produce the phenotype, suggesting that an enhanced activation of both IL-6 and IL-6R is required for cardiac hypertrophy. Therefore, in the present study, one might speculate that gp130 gene expression would be increased in the failing heart. This third question will necessitate further studies. It should also be noted that in addition to activation of CT-1 in cardiomyocytes, a role for CT-1 secretion from cardiac fibroblasts has recently been reported.

In summary, the present study demonstrates that CT-1 gene expression and immunoreactivity are present in the normal canine heart. Importantly, we report that cardiac CT-1 gene expression and immunohistochemical staining are augmented in experimental CHF. Last, we report an important positive relationship between ventricular CT-1 gene expression and left ventricular hypertrophy in CHF, suggesting that CT-1 may play an important role in the structural remodeling that characterizes CHF.

Acknowledgments

This work was supported by grants from the American Heart Association Minnesota Affiliate (MN-97-GB-06), the National Institutes of Health (HL-36634), the Miami Heart Research Institute, the Bruce and Ruth Rappaport Program in Vascular Biology, and the Mayo Foundation. We acknowledge the technical assistance of Denise M. Heublein and Sharon M. Sandberg.

References


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_Circulation_. 2000;101:14-17
doi: 10.1161/01.CIR.101.1.14
_Circulation_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7322. Online ISSN: 1524-4539

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