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Alpha-calcitonin gene-related peptide is protective against pressure overload-induced heart failure

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ABSTRACT

The sensory neuropeptide, α-calcitonin gene-related peptide (α-CGRP) is protective against hypertension-induced heart damage and cardiac ischemia/reperfusion injury. To determine whether this neuropeptide is also cardioprotective in heart failure, this study examined whether the absence of α-CGRP exacerbated the adverse cardiac remodeling, dysfunction and mortality in pressure overload heart failure induced by transverse aortic constriction (TAC). Male α-CGRP knockout (KO) and wild type (WT) mice had TAC or sham surgery at day 0 and were studied on days 3, 14, 21, and 28. The survival rate of TAC α-CGRP KO mice was lower than the TAC WT mice over the duration of the protocol. Left ventricular α-CGRP content in TAC WT mice was higher at days 3, 14, and 21 than sham WT mice. Echocardiography demonstrated greater adverse cardiac remodeling and dysfunction in the TAC α-CGRP KO compared to the TAC WT mice. The lung/body weight ratios and left ventricular masses were higher in TAC α-CGRP KO compared to the TAC WT mice. While there was increased cardiac fibrosis in the TAC WT mice compared to shams, the TAC α-CGRP KO mice had markedly increased fibrosis above that of the TAC WT mice. TAC WT mice had greater cardiac inflammation, cell death, and adaptive angiogenesis compared to sham mice. Importantly, the TAC α-CGRP KO mice had greater inflammation, cell death, and attenuation of angiogenesis compared to TAC WT hearts. Thus, α-CGRP plays a significant protective role in TAC-induced heart failure which may be mediated by decreased inflammation, cell death, and fibrosis.

1. Introduction

Calcitonin gene-related peptide (CGRP) is derived from the tissue-specific splicing of the primary transcript of the calcitonin/α-CGRP gene [1]. CGRP synthesis is limited almost exclusively to specific regions of the central and peripheral nervous systems. There is a CGRP homolog (β-CGRP) that is produced by its own gene [1–3]. The two CGRP genes, α- and β-CGRP share >90% homology and have similar biological properties. However, α-CGRP is by far the predominant product in dorsal root ganglia (DRG) sensory neurons and appears to be the CGRP gene product that plays a significant role in the regulation of cardiovascular function [1–3].

In the periphery α-CGRP is found primarily in sensory neurons that project capsaicin sensitive C-fibers and Aδ-fibers that are usually closely associated with blood vessels [1–3]. Sensory nerves diffusely and prominently innervate the heart between myofibrils and are located in the coronary vasculature. These locations make them perfectly situated to sense and respond to changes in blood pressure, ischemia, and cytotoxic stress in the heart. We and others have demonstrated that α-CGRP acts as a compensatory depressor to attenuate the rise in blood pressure in multiple rat models of experimental hypertension [4–7]. In addition, we have used the α-CGRP KO mouse to demonstrate that α-CGRP is protective against deoxycorticosterone-salt (DOC-salt) hypertension induced heart and kidney damage [1,8] and cardiac ischemia/reperfusion (I/R) injury [9]. Other investigators, using primarily pharmacological approaches, have observed a cardioprotective role for CGRP in cardiac ischemia/reperfusion (I/R) injury [10], myocardial infarction [10], and preconditioning [11]. However, the mechanism(s) that mediate the protective activities of α-CGRP are not well defined.

Several lines of evidence indicate that α-CGRP may have cardioprotective activity against heart failure. Plasma levels of CGRP and the CGRP receptor are elevated in human heart failure [12]. Acute infusion of CGRP to patients with heart failure markedly improves hemodynamic parameters and symptoms [13–15]. In addition, animal studies indicate that α-CGRP benefits the heart at several different levels. For example CGRP: (1) increases cardiac blood flows through its potent vasodilator activity thereby enhancing cardiac performance [1,16]; (2) protects the heart from ischemic and metabolic stress [10];

Abbreviations: CGRP, calcitonin gene-related peptide; TAC, transverse aortic constriction.

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(3) decreases angiotensin II activity [17]; and (4) modulates the immune system by increasing anti-inflammatory cytokine expression while suppressing pro-inflammatory cytokines and chemokines [10]. Despite the well-established protective effects of CGRP in the cardiovascular system, the mechanism(s) that mediate the protective activities of α-CGRP are not well defined. Accordingly, there is a need for studies that are focused on the effects of α-CGRP on the underlying mechanisms associated with pathological cardiac remodeling. Therefore, α-CGRP KO and WT mice were subjected to TAC-induced pressure overload or sham-surgery to determine whether the absence of α-CGRP increases the adverse cardiac remodeling, dysfunction, and death in heart failure.

2. Methods

2.1. Pressure overload model

The animal protocols were approved by the institutional Animal Care and Use Committee (protocol 2079) and are in accordance with the National Institutes of Health *Guidelines for the Care and Use of Laboratory Animals*. The α-CGRP/CT global KO mice were generated and generously provided by Dr. Robert F. Gagel (University of Texas M. D. Anderson Cancer Center, Houston, TX). Eight to ten week old (26–28 g) male α-CGRP KO mice and their weight matched C57/BL6 counterparts were used in this study. Animals were anesthetized by inhalation of isoflurane (1–3% to effect). The depth of anesthesia is monitored by heart rate, tail pinch, muscular relaxation, ECG, and pedal reflex. The TAC procedure was performed using a 27 gauge needle as described elsewhere [18]. Sham surgeries on α-CGRP KO and WT mice were performed by omitting the actual aortic banding and served as a control for all experimental groups. Euthanasia is performed by deep anesthesia (4% isoflurane) followed by removal of the heart and lungs.

2.2. Transthoracic echocardiography

Echocardiography is performed using Vevo 770 High-Resolution Imaging System with a 37.5-MHz high-frequency linear transducer (VisualSonics Inc. Toronto, ON, Canada). Mice were sedated with isoflurane and the heart rate was maintained at 430–480 beat per minute by adjusting isoflurane (~1%). Short-axis M-model images were recorded at the papillary muscle level. Left ventricular internal diameter at end-systole (LVIDs), end-diastole (LVIDd), left ventricular posterior wall thickness, end-systole (LVPWs) and end-diastole (LVPWd) were measured. Fractional shortening (FS) was calculated by VisualSonics Measurement Software using the standard formula.

2.3. Morphometrics and histopathology

At the experimental time points the mice were weighed and anesthetized by isoflurane. Hearts and lungs were removed and the wet weight of the hearts (HW) and wet lungs (LW) were measured as indices of cardiac hypertrophy and left ventricle (LV) function. The basal portion of the LV was fixed in 4% para-formaldehyde and the apical portion was snap frozen in liquid nitrogen and stored at −80 °C for subsequent analysis.

The fixed samples were embedded in paraffin and serial 5 μm cross sections were obtained. The sections were stained with hematoxylin–eosin (H&E) for assessment of myocardial morphology. The average cardiomyocyte cross sectional area (CSA) was calculated from the area of 50 cardiomyocytes measured by ImageJ (NIH). LV collagen volume fraction was determined by Masson Trichrome staining. Twenty representative microscopic fields (200 ×) and 10 arterials (>50 μM) per section were imaged. The percentage of the fibrosis was quantified by using Image-Pro plus software (Media Cybernetics, Inc., Bethesda, MD). A combination of collagen staining with corresponding H&E stained foci from each section was used to assess necrosis.

LV macrophage infiltration was detected by immunostaining heart sections with rat anti-mouse macrophage Mac-2 antibody (Cedarlane, Burlington, NC), quantitated with Image-Pro software and presented as the number of macrophages per mm^2. LV capillary density was measured by immunostaining with antibody against platelet and endothelial cell adhesion molecule-1 (PECAM1, Santa Cruz Biotechnology, Santa Cruz, CA). The Tyramide Signal Amplification kit (Perkin Elmer, Boston, MA) was used for staining. The number of PECAM-1 stained microvessels was obtained and the microvessel density was calculated.

A TUNEL labeling kit was used according to manufacturer's instruction (Roche, Mannheim, Germany) for detection of LV apoptotic cardiomyocytes. The number of apoptotic cardiomyocytes was determined in 20 fields (400×) using Image-Pro plus software, and expressed as apoptotic cardiomyocytes per mm^2.

2.4. Western blotting and measurement of LV α-CGRP

Total protein from LV tissue was extracted by T-PER tissue protein extraction reagent (Thermo Scientific, Rockford, IL). Protein samples were fractionated by SDS-PAGE for detection of hypoxia inducible factor-1 alpha (HIF1α), vascular endothelial growth factor (VEGF), fibroblast growth factor-2 (FGF2), and TNF-α, respectively, then transferred to nitrocellulose membranes. The membranes were incubated with corresponding antibodies against HIF1α (Novus Biologicals, Littleton, CO), VEGF, FGF2, TNF-α and β-actin (Santa Cruz Biotechnology,

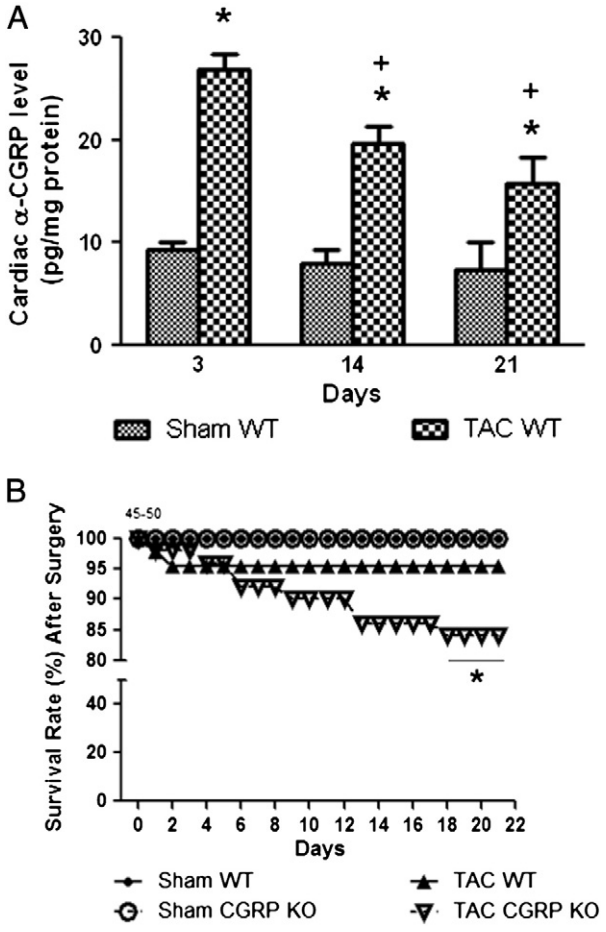

Fig. 1. Increased LV α-CGRP content and survival rate in WT mice following TAC. A. LV α-CGRP levels in TAC and sham WT mice are expressed as the mean ± SEM (n = 6/group). *p < 0.001 sham WT, +p < 0.05 vs TAC WT at day 3. B. Survival rate after TAC (50 α-CGRP KO and 45 WT mice) or sham surgery (42 each CGRP KO and WT mice). *p < 0.05 vs TAC WT mice.