Biomedicine & Pharmacotherapy 99 (2018) 261–270

Contents lists available at ScienceDirect

Biomedicine & Pharmacotherapy

journal homepage: www.elsevier.com/locate/biopha

Genetic ablation of TRPV1 exacerbates pressure overload-induced cardiac hypertrophy

Beihua Zhong[a], Jack Rubinstein[d], Shuangtao Ma[a], Donna H. Wang[a,b,c,*]

[a] Division of Nanomedicine and Molecular Intervention, Department of Medicine, Michigan State University, East Lansing, MI, USA
[b] Neuroscience Program, Michigan State University, East Lansing, MI, USA
[c] Cell & Molecular Biology Program, Michigan State University, East Lansing, MI, USA
[d] Division of Cardiovascular Diseases, Department of Internal Medicine, University of Cincinnati College of Medicine, Cincinnati, OH, USA

ARTICLE INFO

Keywords:
TRPV1
Heart
Hypertrophy
Inflammation
Macrophage

ABSTRACT

Transient receptor potential vanilloid 1 (TRPV1) channels expressed in sensory nerves may regulate vascular tone and cardiovascular function via their anti-inflammatory effects by releasing neuropeptide calcitonin gene-related peptide (CGRP). Inflammation plays a role in the progression of cardiac hypertrophy and TRPV1 activation may be key to cardiac inflammatory processes. The aim of this study was to test the hypothesis that TRPV1 modulates inflammatory processes to protect the heart from pressure overload-induced hypertrophy and inflammatory responses. Trpv1 knockout (*Trpv1*$^{-/-}$) and wild-type (WT) mice were subjected to transverse aortic constriction (TAC) or sham operation. Four weeks after TAC, WT and *Trpv1*$^{-/-}$ mice had developed left ventricular (LV) hypertrophy with increased LV mass, fibrosis and infiltration of macrophages as well as increased secretion of tumor necrosis factor α, interleukin-6 from cardiac tissue (all $P < 0.05$), those were higher in *Trpv1*$^{-/-}$ than in WT mice with TAC (all $P < 0.05$). In addition, decreases of LV ejection fraction and fractional shortening were greater in *Trpv1*$^{-/-}$ than in WT mice (both $P < 0.05$). Moreover, atrial natriuretic peptide level was greater in *Trpv1*$^{-/-}$ than in WT mice with TAC ($P < 0.05$). Compared to sham control, TAC procedure significantly increased cardiac TRPV1 expression and CGRP release in WT mice (both $P < 0.05$), but not in *Trpv1*$^{-/-}$ mice. These results demonstrate that Trpv1 gene deletion results in excessive inflammation, exaggerates cardiac hypertrophy, and deteriorates cardiac function after TAC, which may be due to abnormal cardiac remodeling and decreased CGRP in the absence of TRPV1.

1. Introduction

Cardiac hypertrophy is a compensatory response to pressure overload, volume stress, and myocardial infarction, which is a major risk factor for the progressive development of heart failure. Pressure overload leads to myocardial hypertrophy, cardiomyocytes apoptosis, inflammatory responses, cardiac fibrosis and subsequent ventricular dysfunction [1,2]. Inflammatory cytokines, such as tumor necrosis factor α (TNFα), interleukin 6 (IL-6), and activation of nuclear factor-κB (NF-κB) play a key role in the development of cardiac hypertrophy and remodeling [3–5]. In addition, mice with overexpression of TNFα develop hypertrophic cardiomyopathy [6]. Therefore, inhibiting the inflammatory response and suppressing the release of inflammatory cytokines may provide beneficial effects on cardiac hypertrophy.

The transient receptor potential vanilloid 1 (TRPV1) receptor is a ligand-gated nonselective cation channel, mainly expressed in primary sensory neurons and sensory C- and Aδ-fibers, which can be activated by both physical and chemical stimuli including noxious heat, protons, vanilloid compounds such as capsaicin, lipid metabolites including endogenous ligands *N*-arachidonoyldopamine (NADA) and *N*-oleoyldopamine (OLDA) [7–9]. Capsaicin and endovanilloids, such as NADA and OLDA, possess immunosuppressive activity. For example, NADA inhibits the activation of NF-κB, nuclear factor of activated T-cells (NFAT), and activator protein 1 signaling pathways [10,11]. Activation of TRPV1 increases the level of anti-inflammatory cytokine IL-10 and attenuates the increase in pro-inflammatory cytokines, including TNFα and IL-6, in septic rats [12]. Therefore, TRPV1 may through its anti-inflammatory effects provide beneficial action against cardiac remodeling and hypertrophy. It has been well documented that TRPV1 may regulate vascular tone and cardiovascular function via its anti-inflammatory effects in a variety of conditions such as hypertension, endotoxic shock, and myocardial ischemia reperfusion injury [13–15]. TRPV1 ablation aggravates inflammatory response, enhances cardiac fibrosis, exaggerates progression of left ventricular (LV) remodeling,

* Corresponding author at: Division of Nanomedicine and Molecular Intervention, Department of Medicine, Michigan State University, 788 Service Rd., East Lansing, MI 48824, USA.
E-mail address: donna.wang@hc.msu.edu (D.H. Wang).

https://doi.org/10.1016/j.biopha.2018.01.065
Received 28 September 2017; Received in revised form 18 December 2017; Accepted 11 January 2018

and deteriorates cardiac function following acute myocardial infarction [16]. TRPV1 exerts the cardiovascular protective function via, at least in part, the release of sensory neuropeptides including substance P (SP) and calcitonin gene-related peptide (CGRP), which may mediate vasodilatation as well as be involved in the regulation of inflammation [17,18]. Furthermore, the myocardium and the coronary system possess dense capsaicin-sensitive sensory nerve innervation and TRPV1 expression, suggesting that TRPV1 may exert a strong influence on heart function, cardiac reflexes and adaptive response [13,19]. Studies have shown that TRPV1 plays a protective role in cardiac ischemic stress [16,20,21] and cardiac sensory nerves play a protective role in doxorubicin-induced heart failure [22]. Moreover, cardiac sensory nerves strongly influence the gene expression levels in the heart [23]. However, it is unknown whether TRPV1 plays a role in the cardiac inflammatory process under the condition of transverse aortic constriction (TAC)-induced pressure overload, and if so, how it affects cardiac remodeling and hypertrophy. This study tests the hypothesis that TRPV1 modulates inflammatory processes to protect the heart from pressure overload-induced hypertrophy and functional deterioration.

2. Materials and methods

2.1. Aortic banding

Male Trpv1 gene knockout (*Trpv1*$^{-/-}$) strain B6.129 × 1-Trpv1^{tm1Jul}/J and genetic background-matched control wild type (WT) strain C57BL/6J mice were used (Jackson Laboratory, Bar Harbor, Maine). The experiments were approved by the Institutional Animal Care and Use Committee of Michigan State University. Seven- to nine-week-old male mice were anesthetized with a mixture of ketamine (100 mg/kg, i.p.) and xylazine (8 mg/kg, i.p.). TAC surgery was performed as described by Hu et al. [24]. In brief, the aortic arch was exposed in spontaneously breathing mice. A 6-0 silk thread was passed under the aorta and a bent 27-gauge needle was placed alongside the aortic arch. After ligation, the needle was quickly removed, the skin was closed, and the mice were allowed to recover on a heating pad set at 37 °C. Sham mice underwent the same procedure without ligation of the aorta.

2.2. Transthoracic echocardiography

After four weeks of TAC, mice were anesthetized with pentobarbital (50 mg/kg, i.p.). Echocardiography was performed using a GE Vivid 7/Vingmed ultrasound machine (General Electric) with a 10-MHz transducer applied parasternally to the chest wall. The following parameters were measured as indicators of cardiac function and remodeling: LV internal diameter in diastole (LVIDD) and in systole (LVIDS), posterior wall thickness in diastole (LVPWD) and in systole (LVPWS), LV ejection fraction (EF), and fractional shortening (FS) were directly calculated with integrated software.

2.3. Histological analysis and immunostaining

LV tissue was obtained, fixed in 10% formaldehyde for 24 h, and embedded in paraffin. Serial sections at 5μm in thickness were cut and stained with hematoxylin & eosin for the evaluation of cell morphometry, with collagen-specific Picrosirius red for the detection of fibrosis, or were prepared for immunohistochemistry analysis. Rat anti-mouse Mac-2 antibody (1:100, Cedarlane) and biotinylated rabbit anti-rat secondary antibody (1:100, Vector Laboratories) were used for immunohistochemistry staining of macrophages. All the quantitative analyses were performed by 2 independent investigators on blind specimens. Macrophages were counted in stained sections. To measure the myocyte cross-sectional area, each section was photographed and the cross-sectional area of 150 cells per sample was averaged. The inter-observer variabilities (5 determinations of the same sections on 5 different days) for myocyte cross-sectional diameter were 6.3%.

2.4. Hydroxyproline assay for collagen content

The collagen content of the myocardial tissue was determined by the hydroxyproline assay [25].The absorbance was measured at 565 nm, and hydroxyproline levels were calculated using a standard curve made with 4-hydroxy-1-proline and expressed as micrograms per gram of tissue.

2.5. Determination of cardiac cytokines TNFα and IL-6 secretion by ELISA assay [26]

At four weeks after operation, mice were sacrificed and LV slices (0.5 mm in thickness) were transferred immediately to the culture medium containing RPMI 1640, 25 mM HEPES, 5% fatal calf serum, 30 mM mercaptoethanol, 0.57 mM ascorbic acid, 1.3 mM calcium and 100 IU/ml penicillin and saturated with 95% O_2 − 5% CO_2 at 37 °C for 60min balance, the second hour aliquots were collected and immediately frozen at −80 °C until measurements [26]. Preliminary results showed the concentration of lactate dehydrogenase remained stable during the experiment period, indicating the vital tissue (data not shown). TNFα and IL-6 release from cardiac tissue were measured according to the manufacture's protocol by using commercially available mice-specific ELISA kits (R&D Systems, Minneapolis, MN).

2.6. Western blot

LV tissue was homogenized in lysis buffer containing protease inhibitors cocktail (Sigma). Twenty micrograms of membrane protein sample were separated on an 8%–10% sodium dodecyl sulfate-polyacrylamide gel and transferred to a polyvinylidene difluoride membrane. Blots were blocked 2 h at room temperature in 5% BSA (bovine serum albumin) in washing solution (TBS, 50 mmol/L Tris-HCl, 100 mmol/L NaCl and 0.1% Tween-20 at pH 7.5). Subsequently, blots were incubated overnight at 4 °C with anti-TRPV1 (1:2000) and anti-atrial natriuretic peptide (ANP, 1:1000, Santa Cruz Biotechnology) antibodies in 5% BSA in TBS with gentle shaking, followed by incubation membrane with appropriate horseradish peroxidase-conjugated secondary antibodies (1:2000 or 1:1000, Santa Cruz Biotechnology) at room temperature for 1 h. Proteins were visualized with an enhanced chemiluminescence detection system (ECL, Amersham Biosciences, Piscataway, NJ). The densities of specific bands were determined using Image J 1.46 (NIH), and the TRPV1 and ANP protein levels were expressed relative to levels of GAPDH.

2.7. Quantitative real-time PCR

Total RNA was isolated using the TRIzol reagent (Invitrogen). cDNA was synthesized from 1μg of total RNA using a reverse transcription kit (TaKaRa). Quantitative real-time PCR was performed in triplicate with the Takara SYBR premix (TaKaRa). The relative amount of mRNA was calculated by $2^{-\Delta\Delta CT}$ and was normalized to a housekeeping gene Gapdh. PCR primer sequences: Tgfb1: F, 5′-ACT ACT ATG CTA AAG AGG-3′, R, 5′-TTG TTG CTA TAT TTC TGG-3′; Col1a1: F, 5′-TCA AGG TCT ACT GCA ACA TGG-3′, R, 5′-AAT CCA TCG GTC ATG CTC TCT-3′; Col3a1: F, 5′-CTG GTT CTT CTG GAC ATC C-3′, R, 5′-TCT TCC TGA CTC TCC ATC C-3′; Cd68: F, 5′-TAC ATG GCG GTG GAG TAC AA-3′, R, 5′-AGG TGG ACA GCT GGT GAA AG-3′; Tnf: F, 5′-CCC CAA AGG GAT GAG AAG TT-3′, R, 5′-CAC TTG GTG GTT TGC TAC GA-3′; Il6: F, 5′-AAC GAT GAT GCA CTT GCA GA-3′, R, 5′-GGT ACT CCA GAA GAC CAG AGG A-3′; Gapdh: F, 5′-AGG TCG GTG TGA ACG GAT TTG-3′, R, 5′-TGT AGA CCA TGT AGT TGA GGT CA-3′.

2.8. Measurement of CGRP level in plasma

Mice were sacrificed at four weeks after TAC or sham operation. The plasma was collected and stored at −80 °C for later measurement.