Induced Overexpression of Na^+/Ca^{2+} Exchanger Does Not Aggravate Myocardial Dysfunction Induced by Transverse Aortic Constriction

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ABSTRACT

Background: Alterations in expression and activity of cardiac Na^+/Ca^{2+} exchanger (NCX1) have been implicated in the pathogenesis of heart failure.

Methods and Results: Using transgenic mice in which expression of rat NCX1 was induced at 5 weeks of age, we performed transverse aortic constriction (TAC) at 8 weeks and examined cardiac and myocyte function at 15−18 weeks after TAC (age 23−26 weeks). TAC induced left ventricular (LV) and myocyte hypertrophy and increased myocardial fibrosis in both wild-type (WT) and NCX1-overexpressed mice. NCX1 and phosphorylated ryanodine receptor expression was increased by TAC, whereas sarco(endo) plasmic reticulum Ca^{2+}-ATPase levels were decreased by TAC. Action potential duration was prolonged by TAC, but to a greater extent in NCX1 myocytes. Na^+/Ca^{2+} exchange current was similar between WT-TAC and WT-sham myocytes, but was higher in NCX1-TAC myocytes. Both myocyte contraction and $[Ca^{2+}]_i$ transient amplitudes were reduced in WT-TAC myocytes, but restored to WT-sham levels in NCX1-TAC myocytes. Despite improvement in single myocyte contractility and Ca^{2+} dynamics, induced NCX1 overexpression in TAC animals did not ameliorate LV hypertrophy, increase ejection fraction, or enhance inotropic (maximal first derivative of LV pressure rise, +dP/dt) responses to isoproterenol.

Conclusions: In pressure-overload hypertrophy, induced overexpression of NCX1 corrected myocyte contractile and $[Ca^{2+}]_i$ transient abnormalities but did not aggravate or improve myocardial dysfunction. (*J Cardiac Fail 2013;19:60−70*)

Key Words: Tetracycline-off, fura-2, in vivo catheterization, intracellular Ca^{2+} regulation.

The cardiac Na^+/Ca^{2+} exchanger (NCX1) mediates Ca^{2+} efflux and influx during an action potential (AP) and is therefore intimately involved with regulation of intracellular Na^+ ($[Na^+]_i$) and Ca^{2+} ($[Ca^{2+}]_i$) concentrations during excitation-contraction (EC).[1] Overexpression[2,3] and down-regulation[4] of NCX1 in adult rat left ventricular (LV) myocytes in primary culture result in changes in myocyte contraction and $[Ca^{2+}]_i$ transient amplitudes. Specifically, at low (0.6 mmol/L) extracellular Ca^{2+} concentrations ($[Ca^{2+}]_o$), conditions that favor Ca^{2+} efflux via forward Na^+/Ca^{2+} exchange, overexpression[3] and down-regulation[4] of NCX1 resulted in contraction and $[Ca^{2+}]_i$ transient amplitudes that are lower and higher, respectively, compared with their respective controls. Conversely, at high $[Ca^{2+}]_o$ (5 mmol/L), conditions that favor Ca^{2+} influx via reverse Na^+/Ca^{2+} exchange, contraction and $[Ca^{2+}]_i$ transient

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amplitudes are higher in NCX1-overexpressed but lower in NCX1−down-regulated myocytes compared with their respective controls. At physiologic $[Ca^{2+}]_o$ (1.8 mmol/L), both contraction and $[Ca^{2+}]_i$ transient amplitudes are similar in NCX1-overexpressed or−down-regulated myocytes compared with their respective controls. In the intact heart, homozygous (Hom) but not heterozygous (Het) mice constitutively overexpressing NCX1 exhibit impaired LV fractional shortening,[5] and cardiac-specific knockout of NCX1 results in modest diminution of global LV function.[6]

We have previously generated a novel transgenic (TG) mouse model in which expression of rat NCX1 TG is under the control of a cardiac-specific promoter driving the expression of a tetracycline transactivator (tTA).[7] When doxycycline (Dox) is removed from the feed at 5 weeks of age, expression of NCX1 TG is induced (Ind), resulting in NCX1 protein levels ∼2.5 times that present in wild-type (WT) or noninduced (non-Ind) hearts, without changes in expression of other proteins involved in EC coupling. Compared with WT or non-Ind myocytes, Ind myocytes exhibit ∼42% higher NCX1 current (I_{NaCa}) amplitude, ∼2-fold prolongation of action potential duration, and contraction and $[Ca^{2+}]_i$ transient amplitudes that are lower at 0.6, not different at 1.8, and higher at 5.0 mmol/L $[Ca^{2+}]_o$. Cardiac function, as evaluated by in vivo closed-chest catheterization and echocardiography, is similar among WT, non-Ind, and Ind mice. The cardiac phenotype of Ind mice is similar to Het mice constitutively overexpressing NCX1.

Alterations in NCX1 expression and/or activity have been observed in many models of cardiac hypertrophy and heart failure.[8] It remains controversial, however, whether increases in NCX1 expression and activity is a beneficial compensatory mechanism in response to contractile dysfunction, or is detrimental, leading to progressive heart failure. One approach to differentiate whether increased NCX1 expression is ''friend or foe'' is the ability to ''switch on'' the expression of NCX1 TG concomitantly with the onset of disease state, and evaluate subsequent cardiac performance. In the present study, we tested the hypothesis that switching on NCX1 TG expression shortly before transverse aortic constriction (TAC) is beneficial to myocyte and myocardial contractility.

Methods

Generation of Inducible NCX1 TG Mouse and Transverse Aortic Constriction Surgery

Details of inducible NCX1 TG mouse generation and its characterization have been published.[7] Briefly, rat NCX1 gene[3] was cloned into a cardiac-specific and inducible controlled vector (TREMHC) composed of a modified mouse α-myosin heavy chain (α-MHC) minimal promoter fused with nucleotide-binding sites for tTA.[9] NCX1 TG mice engineered on FVB background were crossed with cardiac tTA TG mice in FVB background (MHC-tTA). Littermates that were heterozygous for tTA but negative for NCX1 TG were used as WT controls. In this ''tetracycline-off'' inducible system, Dox (300 mg/kg mouse diet; Bio-Serv) inhibits tTA transactivation. To induce NCX1 TG expression in adult mice, Dox was removed from the feed at 5 weeks of age. Mice in which the rat NCX1 TG was induced to be expressed are referred to as NCX1 mice throughout this report.

Details of the TAC operation have been published.[10] Briefly, at 8 weeks of age, mice were anesthetized to a surgical plane with tribromoethanol/amylene hydrate (Avertin; 2.5% wt/vol, 8 μL/g intraperitoneally). After intubation with a blunt 20-gauge needle and connection to a volume-cycled rodent ventilator (120 breaths/min) on supplemental oxygen (1 L/min), a midline cervical incision was made to expose the trachea and carotid arteries. Aortic constriction was performed by tying a 7-0 nylon suture ligature against a 27-gauge needle. The needle was promptly removed to yield a constriction of ∼0.4 mm in diameter. Sham operation was identical except that the aorta was not tied. Mice were allowed to recover and studies were performed at 15−18 weeks after TAC (age 23−26 weeks). Only male mice were used in the studies.

Mice were housed and fed on a 12:12-h light-dark cycle at the Thomas Jefferson University and Temple University Animal Facilities and were supervised by veterinary staff members. Standard care was provided to all of the mice used for experiments. All protocols applied to the mice in this study were approved and supervised by the Institutional Animal Care and Use Committees at Thomas Jefferson University and Temple University.

Myocardial Histopathology

Hearts were removed from WT-sham, WT-TAC, and NCX1-TAC mice (n = 3, 4, and 5, respectively), fixed in freshly prepared formalin in phosphate-buffered saline solution, processed for paraffin sectioning (6 μm thickness), and stained with Masson trichrome. Ten sections were obtained from the LV free wall of each mouse. Quantification of fibrous areas was performed with Sigma Scan Pro5. The ratio of area affected by fibrosis (blue color) to total cardiac area in each section was calculated and expressed as percentage fibrosis.[11]

Echocardiographic and Hemodynamic Analyses of Cardiac Function

Transthoracic 2-dimensional echocardiography was performed in anesthetized (2% inhaled isoflurane) mice with a 12-MHz probe as previously described.[7,12−14] LV internal diameters at end-diastole (LVIDD) and end-systole (LVIDS) and ejection fraction (EF) were quantified off-line. For in vivo hemodynamic measurements, a 1.4-French micromanometer-tipped catheter (SPR-671; Millar Instruments) was inserted into the right carotid artery and advanced into the LV of lightly anesthetized (Avertin) mice with spontaneous respirations and placed on a heated (37°C) pad.[7,12−14] Hemodynamic parameters, including heart rate and maximal first-time derivative of LV pressure rise (+dP/dt) and fall (−dP/dt), were recorded in closed-chest mode, both at baseline and in response to increasing doses of isoproterenol (Iso; 0.1, 0.5, 1, 5, and 10 ng).[7,12−14]

Isolation of Adult Murine Cardiac Myocytes

Cardiac myocytes were isolated from the LV free wall and septum of WT and NCX1 mice according to the protocol of Zhou et al[15] and modified by us.[7,12−14,16,17] In all experiments, myocytes were used within 2−8 h of isolation.

Myocyte Shortening Measurements

Myocytes adherent to laminin-coated coverslips were bathed in 0.7 mL air- and temperature-equilibrated (37°C), HEPES-

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