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Junctin is a prominent regulator of contractility in cardiomyocytes

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Abstract

Junctin is one of the components of the ryanodine receptor Ca release channel complex in sarcoplasmic reticulum. To determine the role of acute alteration of junctin protein levels on cardiomyocyte contractility, we used adenoviral-mediated gene transfer techniques in adult rat cardiomyocytes. Acute downregulation of junctin by 40% resulted in significant increases in cell shortening, rate of contraction ($+dL/dt$), and rate of relaxation ($-dL/dt$). The alteration of contractile parameters was associated with increased Ca transient peak and accelerated Ca decay. However, all these contractile and Ca kinetic parameters were depressed significantly when junctin levels were upregulated by 60%. Importantly, there were no alterations in other Ca-cycling protein levels when junctin levels were either decreased or increased. These findings suggest that junctin plays a prominent role in cardiomyocyte Ca-cycling and contractility.
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Junctin (JCN), a 210-amino acid protein, is a sarcoplasmic reticulum (SR) integral component [1]. This 26-kDa protein contains a cytoplasmic NH_2-terminal segment (residues 1–22), a single transmembrane segment (residues 23–44), and a SR intralumenal COOH-terminal segment (residues 45–210), which contains repeated KEKE motifs that are important for protein–protein interaction [1]. Indeed, in cardiac muscle, junctin forms a quaternary protein complex with the ryanodine receptor (RyR), calsequestrin (CSQ), and triadin in the SR lumen [2]. Junctin has been shown to interact with calsequestrin and triadin, and this interaction depends on the SR luminal Ca concentration [1,2]. Junctin also serves as a linker between CSQ and the RyR [1–3].

Among the RyR quaternary protein complex, CSQ and triadin have been suggested to regulate RyR Ca release in cardiac myocytes [4–7]; however, the functional role of junctin is not currently clear. Although transgenic studies showed that junctin may be involved in the regulation of SR Ca-cycling [8,9], adapted mechanisms or even developmental changes associated with chronic expression of high junctin levels may have contributed to the observed phenotype, masking the direct effects of junctin. Intriguingly, in the hearts of mice overexpressing β-adrenergic receptor 1, junctin was shown to decrease as early as two weeks of age, and preceding the alteration of other Ca-cycling protein levels [10]. Notably, the junctin level progressively declined with age and paralleled the development of cardiac hypertrophy and heart failure in this model [10–12]. Thus, it appears that junctin may be involved in the dysregulation of SR Ca-cycling in heart failure. However, it is unknown whether junctin downregulation may be an adaptive mechanism, managing to rescue the deterioration of cardiac function, or one of the primary causes for inducing cardiac hypertrophy and heart failure. Therefore, we acutely manipulated the levels of junctin expression, using adenoviral-mediated gene transfer, and examined the effects of downregulation or upregulation of junctin on cardiac myocyte contractile function and Ca-cycling. Our findings demonstrate that reduced junctin levels were associated

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with enhanced cardiac contractility and Ca kinetics, while increases in junctin had the opposite effects, in the absence of alterations in other Ca-cycling proteins. Thus, for the first time, we show that contractility may be affected by acute alterations of junctin levels, suggesting that junctin is an important modulator of Ca handling and cardiac function.

Materials and methods

Recombinant adenoviral constructs. Recombinant adenoviruses were developed using the pAdTrack-CMV/pAdEasy-1 system. Briefly, a junctin adenovirus (named as AdsJCN) was constructed by insertion of junctin cDNA between the cytomegalovirus (CMV) enhancer/promoter and the SV40 polyadenylation signal sequence of the shuttle vector pAdTrack-CMV, which also carries a GFP expression cassette (Fig. 1A). Antisense junctin adenovirus (named as AdasJCN) was generated by cloning the junctin cDNA in reverse orientation relative to the CMV promoter (Fig. 1A). The resultant plasmids were linearized and co-transformed into *Escherichia coli* BJ5183 cells with an adenoviral backbone plasmid, pAdEasy-1. Recombinant adenoviral vectors were generated through homologous recombination and selected for kanamycin resistance, and the recombination was confirmed by multiple-restriction endonuclease analyses. Then the linearized recombinant vectors were transfected into HEK 293 cells. AdGFP, which contains GFP cDNA and LacZ cDNA controlled by a separate CMV promoter, was used as control. The recombinant viruses were harvested after 7–10 days. The recombinant viruses were prepared as high-titer stocks and further purified by CsCl banding. Viral particle titration was performed by using plaque assay. Briefly, multiple dilutions of virus were plated out with proper amount of 293 cells. When there were individual plaques observed and evenly distributed, the individual plaque number was counted and the plaque forming units per mL (pfu) were obtained by multiplying the count by the dilution factor.

Preparation and infection of cardiomyocyte. Adult rat ventricular myocytes were obtained from Langendorff-perfused hearts of male Sprague–Dawley rats (~300 mg, Harlan Laboratory) at 37 °C, as described before [13]. Briefly, rats were anesthetized with sodium pentobarbital (50 mg/kg IP) and heparinized (10,000 U/kg IP). The hearts were perfused with modified Krebs–Henseleit buffer (KHB) (in mmol/L: NaCl: 118, KCl: 4.8, Hepes: 25, K_2PO_4: 1.25, $MgSO_4$: 1.25, glucose: 11, taurine: 5, and BDM: 10, pH 7.4) for 5 min. Hearts were then perfused with an enzyme solution, which contained 0.7 mg/mL collagenase type II (263 U/mg), 0.2 mg/mL hyaluronidase, 0.1% BSA, and 25 M Ca, for 10 min. Subsequently, the Ca concentration in the perfusion buffer was raised to 100 M, and perfusion continued for five additional minutes. Finally, ventricular tissue was excised, minced, pipette-dissociated, and filtered through a 240-m screen. Cells were harvested and resuspended in 1.8 mM Ca-KHB with 1% BSA, centrifuged briefly again, and resuspended in ACCT medium consisting of DMEM containing 2 mg/mL BSA, 2 mM L-carnitine, 5 mM creatine, 5 mM taurine, 100 IU/mL penicillin, and 100 μg/mL streptomycin. Cells were then counted and plated on laminin-coated glass coverslips or dishes. After 1–2 h, the dishes were infected with adenoviruses in diluted media, at a multiplicity of infection of 500, for 2 h before addition of suitable volume of culture media. Transfection efficiency, determined by GFP gene expression in the cultured cardiac myocytes under a fluorescence microscope, was consistently >95% by this method (Fig. 1B). Myocyte mechanics and Ca kinetics were recorded after 24-h incubation. To obtain intracellular Ca signals, cells were incubated with the acetoxymethyl ester form of fura-2 (Fura-2/AM; 2 M).

Contractile parameter measurements. Myocytes that adhered to the coverslips were bathed in temperature (37 °C)-equilibrated KHB containing 1 mM Ca for 20 min. The myocyte suspension was then placed in a Plexiglas chamber, which was positioned on the stage of an inverted epifluorescence microscope (Nikon Diaphot 200). Myocyte contraction was field stimulated by a Grass S5 stimulator (0.5 Hz, square waves), and contractions were videotaped and digitized on a computer. A video edge motion detector (Crescent Electronics) was used to measure myocyte length and cell shortening, from which the percent fractional shortening

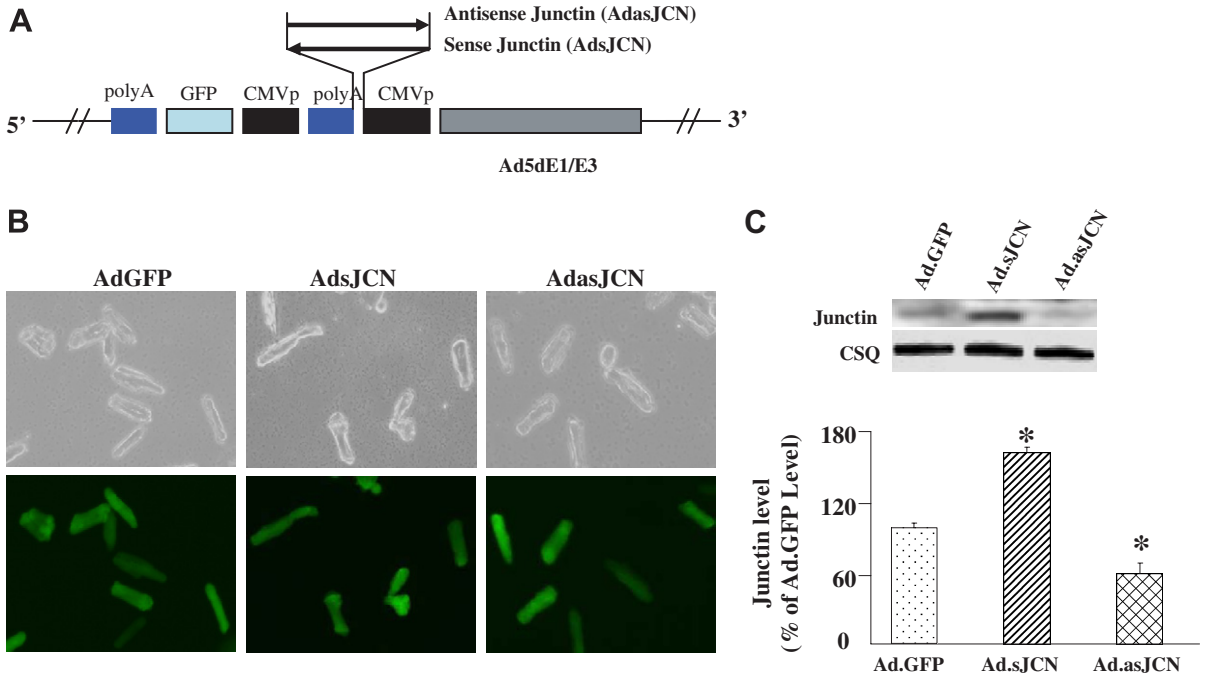

Fig. 1. Generation of recombinant adenovirus vectors: AdsJCN- and AdasJCN-infected, and expression levels of junctin in AdGFP-, AdsJCN-, and AdasJCN-infected cardiac myocytes. (A) Diagram of recombinant adenovirus vectors: AdsJCN- and AdasJCN-infected. (B) AdGFP-, AdsJCN-, and AdasJCN-infected cardiomyocytes under light microscopy (upper panel) and under fluorescence microcopy (lower panel). (C) Immunoblotting analysis of junctin level in AdGFP, AdsJCN, and AdasJCN groups. Protein levels were normalized to that of AdGFP control within the same blot. $n = 3$ hearts in each group. Experiment was repeated three times. Values = means ± SEM. * $P < 0.05$.

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