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Original article

Depressed Frank–Starling mechanism in the left ventricular muscle of the knock-in mouse model of dilated cardiomyopathy with troponin T deletion mutation ΔK210

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ABSTRACT

It has been reported that the Frank–Starling mechanism is coordinately regulated in cardiac muscle via thin filament "on–off" equilibrium and titin-based lattice spacing changes. In the present study, we tested the hypothesis that the deletion mutation ΔK210 in the cardiac troponin T gene shifts the equilibrium toward the "off" state and accordingly attenuate the sarcomere length (SL) dependence of active force production, via reduced cross-bridge formation. Confocal imaging in isolated hearts revealed that the cardiomyocytes were enlarged, especially in the longitudinal direction, in ΔK210 hearts, with striation patterns similar to those in wild type (WT) hearts, suggesting that the number of sarcomeres is increased in cardiomyocytes but the sarcomere length remains unaltered. For analysis of the SL dependence of active force, skinned muscle preparations were obtained from the left ventricle of WT and knock-in (ΔK210) mice. An increase in SL from 1.90 to 2.20 μm shifted the mid-point (pCa_{50}) of the force-pCa curve leftward by ~0.21 pCa units in WT preparations. In ΔK210 muscles, Ca^{2+} sensitivity was lower by ~0.37 pCa units, and the SL-dependent shift of pCa_{50}, i.e., ΔpCa_{50}, was less pronounced (~0.11 pCa units), with and without protein kinase A treatment. The rate of active force redevelopment was lower in ΔK210 preparations than in WT preparations, showing blunted thin filament cooperative activation. An increase in thin filament cooperative activation upon an increase in the fraction of strongly bound cross-bridges by MgADP increased ΔpCa_{50} to ~0.21 pCa units. The depressed Frank–Starling mechanism in ΔK210 hearts is the result of a reduction in thin filament cooperative activation.

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1. Introduction

It is widely accepted that the length dependence of Ca^{2+}-based myofibrillar activation in isolated ventricular preparations underlies the Frank–Starling law of the heart [1,2]. Evidence has been mounting that length-dependent activation is predominantly regulated at the sarcomere level by the giant elastic protein titin (connectin) and thin filament cooperative activation in a coordinated fashion [3], the former of which modulates the distance between the thick and thin filaments (i.e., interfilament lattice spacing) (e.g., [4,5]) and the latter of which determines the fraction of cross-bridges formed upon lattice spacing reduction (e.g., [6,7]).

Dilated cardiomyopathy (DCM) is a disorder characterized by the enlargement of cardiac chambers and systolic/diastolic dysfunctions [8]. Because of a poor prognosis and malignancy of the heart's pump functions, patients with DCM account for as much as ~50% of those who are destined for a heart transplant [9–11]. DCM is caused by mutations in genes for various proteins in cardiomyocytes, including sarcomere proteins, and also by non-genetic insults [8]. Familial DCM constitutes ~30% of all non-ischemic DCM, and various classes of DCM-causing mutations in thin filament regulatory proteins have recently

Abbreviations: DCM, dilated cardiomyopathy; SL, sarcomere length; LV, left ventricle; Tn, troponin; sTn, fast skeletal troponin complex.

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been identified [8,12]. One of the clinically important aspects of the physiology of dilated hearts is the depression of the Frank–Starling mechanism [13–15], which is likely to limit the quality of life for patients with DCM via reduced exercise tolerance. However, the mechanisms of the depressed Frank–Starling mechanism have not been elucidated at the molecular level.

Cardiac muscle contraction is regulated by intracellular Ca^{2+} on a graded basis [16]. At the sarcomere level, actomyosin interaction is regulated by the thin filament-associated troponin (Tn)–tropomyosin (Tm) system [12,17]. Tn is a heterotrimer of distinct gene products: i.e., TnC, TnI and TnT [12,17]. Through interactions with TnC, TnI, Tm and actin, TnT plays a key coordinator function in the Ca^{2+} regulation of the sarcomere [12,17]. Deletion mutation ΔK210 in cardiac troponin T (cTnT) is a recurrent DCM-causing mutation identified worldwide [18], with the disease phenotype highly variable between families despite a monogenic inherited disorder [19]. Therefore, environmental and/or unknown genetic factors are likely to modify the disease phenotype of DCM. In a previous study [20], we created a knock-in mouse model with a DCM-causing deletion mutation ΔK210, and demonstrated that the mutation causes a marked depression in Ca^{2+} sensitivity.

The present study was undertaken to shed light on the mechanistic insights into the relationship between the Frank–Starling mechanism and inherited DCM, by using skinned left ventricular preparations from the knock-in mouse model with the deletion mutation ΔK210 in cTnT.

2. Methods

For a detailed description, see also the Supplemental material.

2.1. Animals

All of the experiments in the present study conformed to the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996). Likewise, all experiments were performed in accordance with the Guidelines on Animal Experimentation of The Jikei University School of Medicine (Tokyo, Japan). Six-week-old wild-type (WT) and *TNNT2*$^{\Delta K210/\Delta K210}$ homozygous knock-in (noted as ΔK210) mice were used throughout the study [20]. In some experiments, six-week-old *TNNT2*$^{+/\Delta K210}$ heterozygous mice were used. All the animals were anesthetized with pentobarbital sodium (50 mg/kg, bolus i.p. administration), and cervical dislocation was performed after loss of eyelid reflex. Thereafter, the hearts were quickly excised from the animals. An increase in heart weight was observed in ΔK210 mice (cf. [20]; Table 1).

2.2. Confocal imaging in the isolated heart

Experiments were performed based on our previous study [21]. Briefly, the heart was perfused with Ca^{2+} free-HEPES-Tyrode's solution containing the plasma membrane stain CellMask™ Orange (1 μM; Life Technologies Inc., Tokyo, Japan) and 80 mM 2,3-butane-dione monoxime. Then, it was mounted on the stage of a microscope (BX-51, Olympus Co., Tokyo, Japan) with a confocal scanning unit (CSU-21, Yokogawa Electric Co., Tokyo, Japan), combined with an objective lens [20× (40×), numerical aperture 1.0 (0.8), Olympus Co.]. The heart was excited at 532 nm, and the resultant fluorescence signals (emission filter: BA575IF, Olympus Co.) were detected by the EMCCD camera (iXon, Andor, Tokyo, Japan). The fluorescence signals were analyzed by using the ImageJ software [21]. Experiments were performed at 30 °C.

2.3. Skinned muscle mechanics

Skinned muscle fibers were prepared from left ventricular papillary muscles according to the method used in our recent studies [6,7].

The force-pCa protocol was performed according to the method described in detail in our recent publications [6,7], at SL 1.90 and then at SL 2.20 μm (SL measured by laser diffraction). Active force was measured at 1.90 μm at various Ca^{2+} concentrations (expressed as pCa), and then at 2.20 μm. Here, the mid-point of the force-pCa curve, i.e., pCa_{50}, reflects the *apparent* dissociation constant (K_d) of Ca^{2+}-binding to troponin C in muscle. In the present study, we used ΔpCa_{50}, i.e., difference between the values of pCa_{50} at SL 1.90 and 2.20 μm, as an index of length-dependent activation because it represents the ratio of the K_d values obtained before and after SL elongation. Force-pCa curves were normalized at pCa 4.5. The steepness of the force-pCa curve was expressed as n_H.

The rate of active force redevelopment (k_{tr}) was measured at maximal and half-maximal activations, as an index of the cross-bridge cycling rate [6].

For the measurement of passive force, skinned preparations were stretched from SL 1.90 μm to 2.20 μm at a constant velocity of 0.1 muscle length/s and held for 30 min, followed by a release to SL 1.90 μm [22].

All the experiments with skinned preparations were conducted at 15 °C to minimize run-down in active and passive forces [6,7].

2.4. Troponin exchange

The Tn exchange was performed with the rabbit fast skeletal Tn complex (sTn) in accordance with our previously published procedure [6,23]. We used sTn in the present study, because in both WT and ΔK210 preparations, cardiac Tn subunits were almost completely replaced by their skeletal counterparts (hence the identical Tn complex in different types of preparations; cf. [6,23]).

2.5. Protein kinase A treatment

The skinned muscle preparations were incubated for 50 min at 25 °C with purified protein kinase A (PKA) at 1 U/μl [22,23].

2.6. Western blotting

Immunoblotting was performed as in our previous reports [21,23].

2.7. Electrophoresis

SDS-PAGE was conducted based on our previous studies [6,23]. We quantified the reconstitution ratio by comparing the ratio of troponin T (TnT) or TnI to actin with that obtained for the original muscle [6,23].

Table 1
Heart weight, lung weight and liver weight of 6-week-old WT and ΔK210 mice. Number of animals, 35 and 32 for WT and ΔK210 mice, respectively. Survival rate, 100% and 71.9% (death, 9 animals) for WT and ΔK210 mice, respectively, at 6 weeks after birth.

	Body weight (g)	Heart weight (mg)	Heart weight/body weight (mg/g)	Lung weight (g)	Liver weight (g)
WT	25.78 ± 0.53	149.12 ± 3.27	5.74 ± 0.086	0.13 ± 0.01	1.22 ± 0.16
ΔK210	23.15 ± 0.57	286.09 ± 13.19*	11.59 ± 0.59*	0.16 ± 0.02	1.42 ± 0.11

* $p < 0.05$.

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