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Original article

Contractile dysfunction of cardiomyopathic hamster myocytes is pronounced under high load conditions

Satoshi Nishimura [a], Hiroshi Yamashita [a], Masayoshi Katoh [a], Kelly P. Yamada [b], Kenji Sunagawa [c], Yasutake Saeki [d], Yosiki Ohnuki [d], Ryozo Nagai [a], Seiryo Sugiura [b,*]

[a] *The Department of Cardiovascular Medicine, Graduate School of Medicine, The University of Tokyo, Tokyo, Japan*
[b] *Computational Biomechanics Laboratory, The Institute of Environmental Studies, Graduate School of Frontier Sciences, The University of Tokyo, Hongo 7-3-1, Bunkyo-ku, Tokyo 113-0033, Japan*
[c] *The Department of Cardiovascular Medicine, Graduate School of Medicine, Kyushu University, Fukuoka, Japan*
[d] *Department of Physiology, Tsurumi University Dental School, Yokohama, Japan*

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Abstract

To understand the pathophysiology of hereditary cardiomyopathy, the contractile function of cardiomyopathic hamsters has been studied at the cellular level. However, most of the studies to date have described the cell shortening under the unloaded condition. Using a novel force–length measurement system for single cardiomyocytes, we studied the contractile function of cardiomyopathic hamster myocytes over a wide range of loading conditions. Cardiomyocytes were isolated from the ventricles of eight- to 10-week-old cardiomyopathic (CMP) hamsters (Bio TO-2 strain), as well as control (CTRL) Syrian hamsters. A pair of carbon fibers was attached to both ends of single cardiomyocytes and their contractile characteristics were recorded while changing the after-load by controlling the fiber motion. Under the unloaded condition, the shortening fraction (CMP 9.2 ± 0.5% vs. CTRL 10.7 ± 0.8%, $P = 0.06$) and maximum shortening velocity (CMP 98.2 ± 7.3 μm/s vs. CTRL 147.2 ± 6.5 μm/s, $P < 0.05$) were decreased in CMP hamster myocytes. The peak force under the isometric condition (CMP 35.8 ± 2.2 mN/mm^2 vs. CTRL 69.0 ± 8.4 mN/mm^2, $P < 0.05$) and external work (CMP 898 ± 130 J/m^3 vs. CTRL 3058 ± 576 J/m^3, $P < 0.05$) under physiologically loaded conditions were also decreased, but the differences were more pronounced under the loaded conditions. Calcium transients measured by Indo-1 revealed elevated diastolic level, decreased peak level, and slower diastolic decay in CMP myocytes thus being consistent with the observed contractile dysfunction. These results clearly indicate the importance of the loading conditions in evaluating the contractile function of CMP hamster myocytes, and may provide insights into the mechanism of contractile dysfunction in this disease.

Keywords: Cardiomyopathic hamster; Bio TO-2 strain; Cardiomyocyte; Isometric force; Calcium transient

1. Introduction

Cardiomyopathic Syrian hamsters have been used as models of hereditary cardiomyopathy and congestive heart failure. Most of the currently available strains are derived from the Bio 14.6 strain, and thus share the common genetic abnormalities identified in the exon of delta-sarcoglycan [1]. However, each strain presents a distinct phenotype depending on the stage of life. For example, some strains show significant cardiac hypertrophy in the early stage of disease (Bio 14.6, UM-X7.1 and CHF 146), whereas others (Bio 53.58 and Bio TO-2) are characterized by prominent dilation of the ventricles without wall hypertrophy [1–3].

To understand the pathogenesis of these animal models and the pathophysiology of heart failure, many studies have been undertaken at the organ [4], tissue [5,6] and cellular [7–11] levels to identify changes in the ion currents [6,11], action potentials [5], calcium kinetics [5,7,10] and mechanical properties [4,6,10]. Among these, studies on mechanics using isolated single cardiomyocytes have the advantage of establishing a direct link between subcellular abnormalities and mechanical properties by eliminating extracellular factors such as fibrosis, which is known to develop in cardiomyopathic tissue. However, most of the studies [7,10,12] in this

* Corresponding author. Tel./fax: +81 3 5841 8393.
E-mail address: sugiura@k.u-tokyo.ac.jp (S. Sugiura).

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field have evaluated the mechanical properties of cardiomyopathic myocytes under unloaded or light load conditions, and are far from the actual situation where the failing heart is working against high after-load and/or preload. Recently, we developed a novel force–length measurement system for isolated single cardiomyocytes using carbon fibers [13,14]. Using this system, in which the motion of the carbon fibers is controlled by a piezo-electric device, we can study the mechanics of a single cardiomyocyte over a wide range of loading conditions, including unloaded, isometric and physiologically loaded conditions.

Accordingly, the purpose of this study was to evaluate the mechanics of single cardiomyocytes isolated from cardiomyopathic hamsters (Bio TO-2 strain) in the early and compensated phases under a wide range of loading conditions. The results clearly revealed that the functional impairment became evident under high load, indicating the importance of functional analysis under loaded conditions. The underlying mechanism for the contractile dysfunction will also be discussed.

2. Materials and methods

2.1. Animals and myocyte isolation

All experiments were conducted in accordance with the National Research Council "Guide for the Care and Use of Laboratory Animals" and approved by our Institutional Animal Care and Use Committee. Eight- to 10-week-old male Syrian cardiomyopathic (CMP) hamsters (Bio TO-2 strain) and age-matched Syrian golden hamsters (control: CTRL) were obtained from the Bio-Breeders Institute (Cambridge, MA). We chose to use the hamsters at this relatively young age because Bio TO-2 strain hamsters are known to show cardiac dysfunction (low cardiac output) at this stage of life without developing significant interstitial fibrosis. The influence of fibrosis on myocyte isolation and functional analysis will be discussed later (Section 4.1).

The hamsters were anesthetized with an intraperitoneal injection of pentobarbital (500 mg/kg body wt.). After anticoagulation with heparin (10,000 U/kg i.p.) the heart was quickly removed and retrograde perfusion was initiated with nominally Ca^{2+}-free HEPES-Tyrode solution (130 mM NaCl, 5.4 mM KCl, 0.5 mM $MgCl_2$, 0.33 mM NaH_2PO_4, 22 mM glucose, 5 mM glutamine, 0.4 mM EGTA, 25 mM HEPES, pH 7.4) at 37 °C. After 5 min, the perfusate was switched to an enzyme solution containing collagenase (1 mg/ml Collagenase Type 2; Worthington), protease (0.05 mg/ml Type XIV; Sigma) and trypsin (0.05 mg/ml; Sigma) and maintained for 20 min. Finally, the enzyme was washed out by perfusion and the calcium concentration of the Tyrode solution was gradually increased to 1.1 mM. The isolated myocytes were then transferred to an experimental chamber, the glass bottom of which was coated with poly-HEME (2-hydroxyethyl methacrylate; Sigma) to prevent adhesion of the myocytes during the force and length measurements.

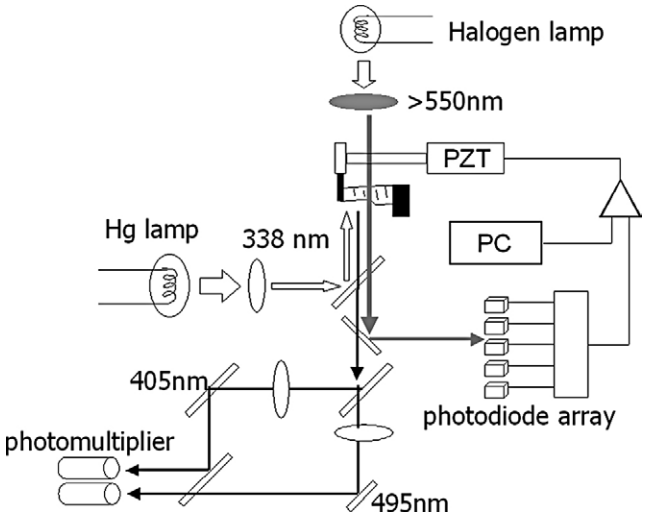

Fig. 1. Diagram of the experimental setup. The position of the fiber is detected by a photodiode array. The position signal is processed by a PC, and the calculated command signal is applied to a piezo-electric translator (PZT) connected to the carbon fiber. To observe the ratiometric Indo-1 signal, the myocytes are illuminated by a high-pressure mercury-arc lamp (excitation 338 nm) and the fluorescent light is divided by a dichroic beam splitter in order to measure two peaks of fluorescence (405 and 495 nm) simultaneously using two photomultiplier tubes.

2.2. Measurement of the force and length relationships

The principle of the single cardiomyocyte force–length measurement system has been described previously [13–15]. Briefly, a single cardiomyocyte was selected under a microscope according to the following criteria: 1) a rod shape with an average sarcomere length of > 1.65 μm (measured by on-line Fourier analysis of optical density traces of the sarcomere pattern of the myocyte image; SarcLen, IonOptix, Milton, MA); and 2) greater than 5% contraction of the total myocyte length in response to electrical stimulation. Next, a pair of carbon fibers was attached to each end of the selected myocyte using micromanipulators. One of the fibers was thin and compliant (diameter 7 μm, stiffness 80–200 nN/μm), while the other was thick and rigid (diameter 30 μm, stiffness > 1000 nN/μm) and served as a mechanical anchor. The image of the compliant fiber was projected onto a linear 1024-element photodiode array (S3903; Hamamatsu Photonics, Japan) to monitor the bending motion induced by active contraction or passive stretching (Fig. 1). Furthermore, we controlled the position of the compliant fiber by moving a piezo-electric translator (PZT) (P-841.40; Physik Instrumente, Germany) that was connected to it. Myocyte length signals obtained by the photodiode array sensor were sampled and processed at 1 kHz, and the generated command signal was applied to the PZT driver with a 16 bit A/D, D/A converter (6035E; National Instruments, TX) connected to a personal computer (PC). The myocyte was electrically stimulated at 0.5 Hz with pulses of 10 ms duration. Before the force measurements, we stretched the diastolic myocyte length to 105% of the slack length while measuring the sarcomere length (IonOptix). All experiments were performed at 37 °C (Thermo-