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High-frequency sarcomeric auto-oscillations induced by heating in living neonatal cardiomyocytes of the rat

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ABSTRACT

In the present study, we investigated the effects of infra-red laser irradiation on sarcomere dynamics in living neonatal cardiomyocytes of the rat. A rapid increase in temperature to >~38 °C induced $[Ca^{2+}]_i$-independent high-frequency (~5–10 Hz) sarcomeric auto-oscillations (*Hyperthermal Sarcomeric Oscillations*; HSOs). In myocytes with the intact sarcoplasmic reticular functions, HSOs coexisted with $[Ca^{2+}]_i$-dependent spontaneous beating in the same sarcomeres, with markedly varying frequencies (~10 and ~1 Hz for the former and latter, respectively). HSOs likewise occurred following blockade of the sarcoplasmic reticular functions, with the amplitude becoming larger and the frequency lower in a time-dependent manner. The present findings suggest that in the mammalian heart, sarcomeres spontaneously oscillate at higher frequencies than the sinus rhythm at temperatures slightly above the physiologically relevant levels.

1. Introduction

It is well established that an increase in body temperature, albeit by a slight magnitude, results in dramatic changes in the functions of various organs, coupled with altered cellular homeostasis. In the heart, an increase in the temperature causes a positive chronotropic effect, coupled with acceleration of the pacemaker activity [1,2].

We previously demonstrated that microscopic heat pulses (ΔT, ~0.2 °C) induced Ca^{2+} transients in HeLa cells [3]. It has likewise been reported that rapid increases in temperature by increments of ~0.5 °C depolarize cellular membranes [4,5]. These previous studies highlight the importance of temperature change by merely <~1 °C in the modulation of cellular functions. In cardiovascular research, recent studies demonstrated that infra-red (IR) laser irradiation induced cardiac beating in various specimens [6–8]), coupled presumably with acceleration of membrane depolarization [4,5]. In contrast to these previous studies focusing on myocardial membrane functions, we demonstrated that an increase in solution temperature from 36 to 41–43 °C (i.e., ΔT, 5–7 °C) induced Ca^{2+}-independent, actomyosin-based isotonic contractions in isolated adult ventricular myocytes of the rat [9].

The state of myocardial sarcomeres depends on the "*on–off*" equilibrium of the thin filament, regulated not only by the intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) but also by strongly bound cross-bridges, in a cooperative fashion [10,11]. Because of the graded nature of the "*on–off*" regulation, cardiac sarcomeres exhibit spontaneous oscillations (SPOCs) at partial activation (i.e., at the non-equilibrium state) when both the force-generating and non-force-generating cross-bridges co-exist over certain threshold populations (i.e., minimum requirement for SPOCs), in the steady-state ionic environment [12].

In the present study, using living neonatal cardiomyocytes, we tested the hypothesis that rapid changes in temperature (within or slightly above the physiologically relevant temperature levels) modulate the state of myocardial sarcomeres via alteration of the thin filament "*on–off*" equilibrium, i.e., relaxation, oscillations and contraction. Experimental findings were analyzed by taking advantage of sarcomere length (SL) nanometry [13].

Abbreviations: F.I., fluorescence intensity; $[Ca^{2+}]_i$, intracellular Ca^{2+} concentration; HSOs, Hyperthermal Sarcomeric Oscillations; SL, sarcomere length; SR, sarcoplasmic reticulum.

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2. Materials and methods

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).

2.1. Microscopic system

Real-time nanoimaging was performed based on our previous studies [13] using an inverted optical microscope (IX-70; Olympus Co., Tokyo, Japan) equipped with an electron-multiplying charge coupled device camera (iXon +897; Andor Technology, Belfast, UK) using a 60× oil immersion objective (N/A, 1.45). A 488 nm laser [FITEL HPU50211 (Blue); Furukawa Electric, Tokyo, Japan] was used for the excitation of AcGFP in the Z-disks of cardiomyocytes, and epi-illumination fluorescence was obtained. As in our previous study [13], we measured the changes in $[Ca^{2+}]_i$ simultaneously with sarcomeric motions, and the myocytes were bathed in a solution containing 2 μM Fluo-8-AM (Dojindo, Kumamoto, Japan) for 20 min at 25 ± 0.5 °C. To obtain the highest possible precision in the SL displacement measurement (i.e., 8 nm in the present study; same level as in our previous study [13] on Fluo-4-loaded myocytes), we performed imaging experiments at a video rate, 33 fps, throughout experimentation.

The temperature of the extracellular solution was controlled with an error of 0.2 °C by a thermostatically controlled incubator on the sample stage (INUG2-ONICS, Tokai Hit, Tokyo, Japan), and measured with a digital thermometer (ASF-250T, AS ONE, Osaka, Japan). The solution was directly heated by focusing the IR laser beam with a wavelength of 1455 nm (KPS-STD-BT-RFL-1455-02-CO, Keopsys, Lannion, France). The changes in temperature were calculated from thermal quenching of the fluorescent dye europium thenoyltrifluoroacetonate trihydrate (Eu-TTA) (Acros Organics, Pittsburgh, PA) coated on the glass-based dish. Eu-TTA (5 mg/ml) and PMMA (10 mg/ml) in acetone were spin-coated on glass-based dishes. The temperature sensitivity of the fluorescence intensity was −2.8% at 25 °C. A mercury lamp (Olympus) and an excitation filter (BP360-370, Olympus) were used for the excitation of Eu-TTA (as in [14]).

2.2. Experimental procedure

Nano-imaging was performed 1 day after pAcGFP-actinin plasmid transfection [13]. In experiments with IR laser irradiation, a cell culture medium [Dulbecco's Modified Eagle Medium: Nutrient Mixture F-12 (DMEM/F-12, HEPES) (Life Technologies) supplemented with 10% fetal bovine serum (Life Technologies), 100 Unit/ml penicillin and 100 μg/ml streptomycin (Life Technologies)] was used as the bathing solution. For experiments on non-beating myocytes, we added 20 mM EGTA, 4 μM thapsigargin (Sigma–Aldrich) and 200 μM ryanodine (Sigma–Aldrich) to the solution, in order to block the sarcoplasmic reticular functions (see [13] and references therein). All the experiments were performed at the basal temperature of 25.0 ± 0.5 °C.

2.3. Data analyses

SL oscillations and $[Ca^{2+}]_i$ were analyzed based on our previous study (see [13] and the Supplementary Material).

2.4. Statistics

Significant differences were assigned using the paired or unpaired *t* test as appropriate. Data are expressed as means ± SEM unless otherwise noted, with *n* representing the number of preparations examined. Linear regression analyses were performed in accordance with the method used in a previous study [13]. Statistical significance was assumed to be $P < 0.05$. N.S. indicates $P > 0.05$.

3. Results and discussion

First, the α-actinin-AcGFP construct produced periodic fluorescence along the longitudinal direction of the neonatal cardiomyocyte, showing AcGFP expression in Z-disks ([13]; Fig. 1A). Infrared (IR) laser irradiation instantaneously increased the solution temperature, as a function of the distance between the laser center and the target point (Fig. 1B). We found that high-frequency sarcomeric auto-oscillations (termed "*Hyperthermal Sarcomeric Oscillations*"; HSOs) were induced upon IR laser irradiation in a reversible and reproducible fashion (Fig. S1A), concurrently with normal beating without detectable wave interference (temperature increased from 25 to ~40 °C in the sarcomere indicated by the yellow arrow in Fig. 1A) (see Movie-1).

The frequency of the change in $[Ca^{2+}]_i$-dependent fluorescence intensity (F.I.) and subsequent beating was ~0.5 Hz before heating, increased to ~1.3 Hz during heating (coupled presumably with acceleration of the excitation–contraction coupling; see [15]) and decreased to ~0.5 Hz following cessation of heating (Fig. 1C). The heating-induced change in the frequency of spontaneous beating was qualitatively consistent with the *in vivo* observation that the heart rate of rat neonates is increased approximately two fold upon increase in temperature from 23 to 33 °C [15]. As shown in the enlarged view of Fig. 1C, HSOs occurred independently of $[Ca^{2+}]_i$ changes, and coexisted with $[Ca^{2+}]_i$-dependent sarcomeric contractions. In the myocyte of Fig. 1A, approximately seven HSOs existed in one cycle of $[Ca^{2+}]_i$-dependent sarcomeric contraction, and on average, SL decreased (or increased) in response to an increase (or a decrease) in $[Ca^{2+}]_i$. Our FFT analyses revealed that in contrast to one peak (~1.1 Hz) for F.I. changes, two distinct peaks of ~1.1 and ~7 to ~10 Hz were present for SL changes, the former corresponding to the F.I. change and the latter to HSOs. A multiple set of data demonstrated that the frequency of Ca^{2+} transient was increased from ~0.5 to ~1.3 Hz upon heating, two times consecutively (Fig. 1D). And the frequency of HSOs remained nearly unchanged at ~10 Hz during heating (Fig. 1D), showing the characteristics of HSOs unaffected by periodic $[Ca^{2+}]_i$ changes.

Next, we investigated whether or not HSOs occurred in myocytes following blockade of the functions of the sarcoplasmic reticulum (SR) (by adding ryanodine (200 μM) and thapsigargin (4 μM) in the solution; [13]). As shown in Fig. 2A, HSOs were induced by heating (from 25 to ~42 °C) in the presence of ryanodine and thapsigargin (see Movie-2), indicating no involvement of the membrane system (i.e., changes in $[Ca^{2+}]_i$) in the occurrence of this phenomenon. Unlike in beating myocytes however, HSOs occurred in a delayed fashion, especially upon the 1st laser irradiation (Fig. S1B), in that the oscillations were clearly observed ~7 s after the onset of heating. Upon the 2nd and 3rd laser irradiation, HSOs appeared ~1 and ~0.5 s after the onset of heating, respectively (Figs. 2A and S1B). As in the myocytes with intact SR functions (Fig. 1), oscillations disappeared upon cessation of heating, consistent with the notion that a rise in temperature directly triggers HSOs.

To confirm that HSOs are indeed a sarcomere-based phenomenon, we tested the effects of the actomyosin inhibitor N-benzyl-p-toluenesulfonamide (BTS, 200 μM) on the oscillations. As shown in Fig. S2, during heating, the single SL displacement was significantly less (~4 times) in the presence of BTS. We previously demonstrated that the heating-induced free shortening of adult cardiomyocytes was blocked by blebbistatin (a myosin II inhibitor) [9].