Biochemical and Biophysical Research Communications 457 (2015) 165–170

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

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

Seine A. Shintani [a], Kotaro Oyama [a], Norio Fukuda [b,*], Shin'ichi Ishiwata [a,c,*]

[a] Department of Pure and Applied Physics, School of Advanced Science and Engineering, Waseda University, Tokyo, Japan
[b] Department of Cell Physiology, The Jikei University School of Medicine, Tokyo, Japan
[c] WASEDA Bioscience Research Institute in Singapore (WABIOS), Singapore

ARTICLE INFO

Article history:
Received 10 December 2014
Available online 27 December 2014

Keywords:
Cardiac muscle
Contractility
Imaging
Sarcomere dynamics

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.

* Corresponding authors at: Department of Cell Physiology, The Jikei University School of Medicine, 3-25-8 Nishi-shinbashi, Minato-ku, Tokyo 105-8461, Japan. Fax: +81 3 3433 1279 (N. Fukuda). School of Advanced Science and Engineering, Waseda University, 3-14-9 Okubo, Shinjuku-ku, Tokyo 169-0072, Japan. Fax: +81 3 5286 3437 (S. Ishiwata).

E-mail addresses: noriof@jikei.ac.jp (N. Fukuda), ishiwata@waseda.jp (S. Ishiwata).

http://dx.doi.org/10.1016/j.bbrc.2014.12.077
0006-291X/

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].

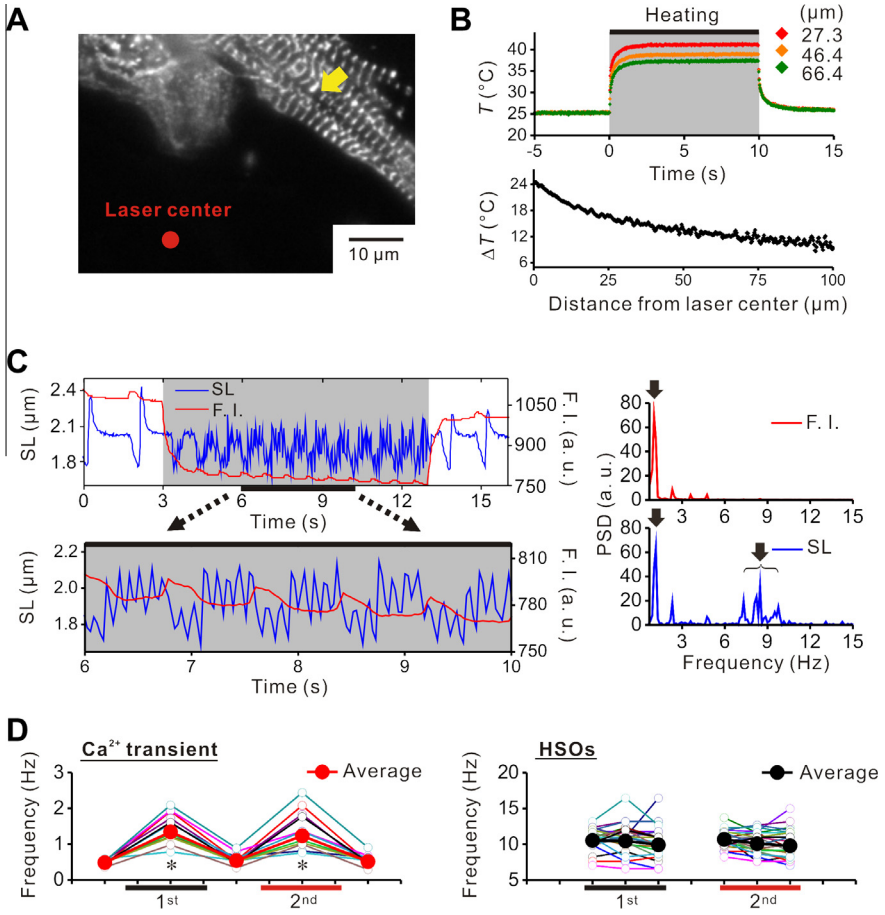

Fig. 1. HSOs in spontaneously beating cardiomyocytes. (A) Epi-illumination image of a myocyte expressed with AcGFP in Z-disks. Fluo-8 loaded into the myocyte. Laser center is indicated (closed red circle). A yellow arrow indicates the sarcomere for the analysis in (C). (B) Top: changes in temperature induced by IR laser irradiation for 10 s. Bottom: relationship of the distance from laser center and ΔT. Given the distance from the laser center, the temperature in the sarcomeres, indicated by the yellow arrow in (A), was estimated to be 39.7 °C. (C) Left, top: time-dependent changes in SL and F.I. in the myocyte in (A) upon IR laser irradiation (2nd irradiation; see Fig. S1A for data of 1st and 2nd irradiations). F.I. was obtained from the whole myocyte in the view window. Heat pulse was given for 10 s, i.e., from 3 to 13 s (in gray). Due to the temperature sensitivity of fluorescence [23], F.I. was decreased (or increased) as heat pulse was given (or ceased). Left, bottom: enlarged view of the graph showing SL on top from 6 to 10 s. HSOs are clearly seen coexisting with $[Ca^{2+}]_i$-dependent spontaneous beating. Right, top: FFT analysis for the changes in F.I. from 4.7 to 12.5 s. Right, bottom: same as in Right, top for the changes in SL. Note that while only one peak is present for F.I., two different components, i.e., slow and fast components, are seen for SL (with the former corresponding to that for F.I.; see arrows). (D) Left: effects of IR laser irradiations on the frequency of Ca^{2+} transient. Closed red circles with error bars, average values; open circles in various colors without error bars, individual data. $^*P < 0.05$ compared with the data before heating (for both 1st and 2nd heating). Right: effect of heating on the frequency of HSOs. Closed black circles with error bars, average values; open circles in various colors without error bars, individual data. The HSO frequency was not significantly changed during the course of heating (both 1st and 2nd heating). Data obtained 1.5, 5 and 8.5 s after the onset of heating were analyzed. $n = 24$ (12 cells).

In an *in vitro* study, Ishiwata demonstrated that an increase in temperature above ~40 °C induces dissociation of the troponin–tropomyosin complex from actin [16,17], suggesting that an increase in temperature promotes the shift of the "*on–off*" equilibrium of the thin filament state toward the "*on*" state and subsequently, the myosin binding to actin via thin filament cooperative activation [10,11]. A recent work by Brunet et al. likewise reported that the sliding velocity of actin filaments (reconstituted with the regulatory proteins troponin and tropomyosin) on the myosin-coated cover glass was increased with an increase in solution temperature [18]. Accordingly, sarcomeric auto-oscillations will be generated when the thin filament activation level becomes partial, viz., under conditions where both force-generating and non-force-generation cross-bridges coexist beyond threshold proportions, based on the mechanism discussed in our previous studies [12,19,20].

We then applied IR laser irradiation to myocytes with blockade of the SR functions, consecutively three times (Fig. S1B), and performed analyses on the waveform properties of HSOs. First, while the changes in maximal SL (at the peak of lengthening) were small in magnitude, minimal SL (at the peak of shortening) became shorter during heating (10 s for each heating; Fig. 2B). As a result of time-dependent enhancement of shortening, the amplitude was increased in a time-dependent manner during heating (Fig. 2C). We also found that saw-tooth oscillations (similar to cell-SPOCs; [13]) appeared as heating continued (see Fig. 2A, bottom, right). In contrast to the amplitude, the frequency was decreased in a time-dependent manner during heating, and it was lowest during the 3rd heating (Fig. 2D). Shortening velocity was slightly decreased during heating, with the magnitude less than that of the amplitude or the frequency (Fig. 2E).

These experimental findings on HSOs can be qualitatively explained by our mathematical model of SPOCs [19,20]. Namely, we previously reported that an increase in the myosin attachment rate (α) results in an increase in the amplitude, as well as a decrease in the frequency [13,19]. Taking into account the findings by Ishiwata [16] as well as those of skinned muscle studies demonstrating the effects of temperature on cross-bridge kinetics [21], it is likely that heating continuously shifts the "*on–off*" equilibrium of the thin filament state toward the "*on*" state (i.e., accelerates the cross-bridge cycling rate), thereby increasing the amplitude of HSOs, coupled with an increase in α Table 1). Consistent with this notion, heating changed the waveform of HSOs from triangular

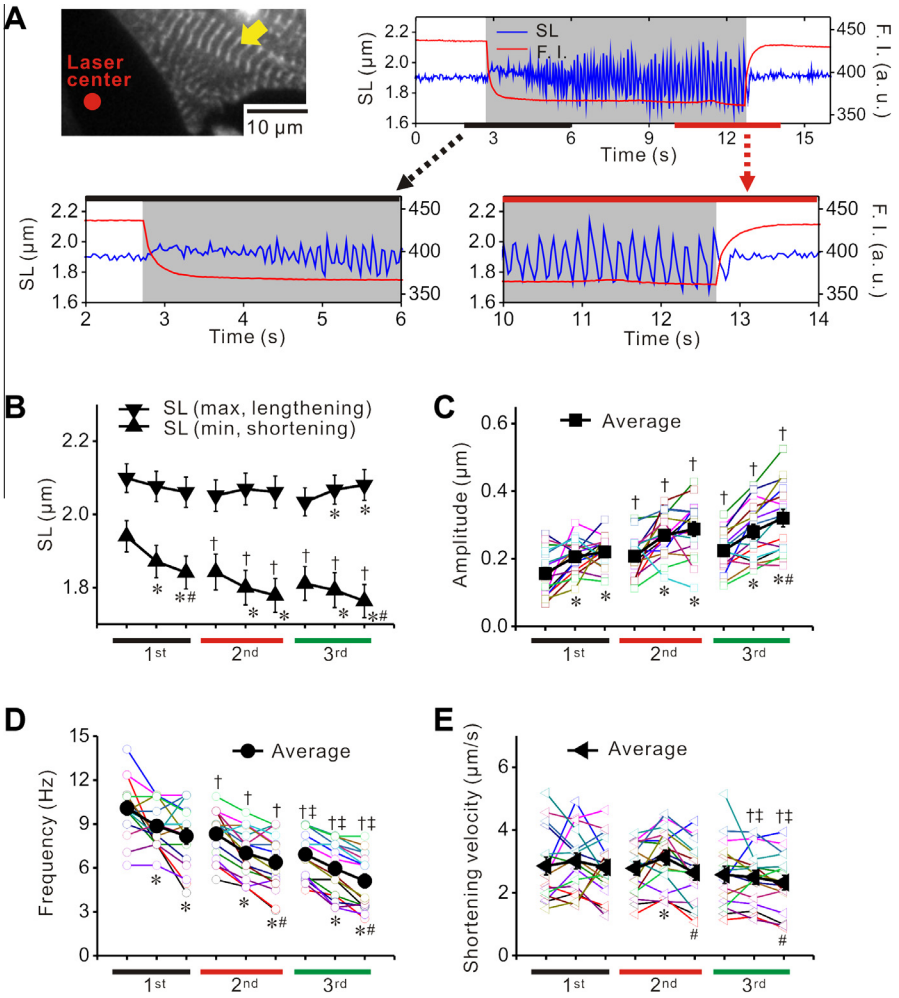

Fig. 2. HSOs in cardiomyocytes with blockade of the SR functions. (A) Top, left: epi-illumination image of a myocyte expressed with AcGFP in Z-disks. Fluo-8 was loaded into the myocyte, and 200 μM ryanodine and 4 μM thapsigargin were present. Laser center is indicated (closed red circle). The yellow arrow indicates the sarcomere used for the analysis. Top, right: time-dependent changes in temperature induced by IR laser irradiation for 10 s (2nd irradiation; see Fig. S1B for data of 1st, 2nd and 3rd irradiations). Given the distance from the laser center, the temperature in the sarcomeres indicated by the yellow arrow in Top, left was estimated to be 40.8 °C. Bottom, left: enlarged view of the graph (2–6 s) showing the occurrence of HSOs. Note that HSOs were induced in a delayed fashion (~1.5 s after the onset of heating). Bottom, right: enlarged view of the graph showing HSOs at the steady-state. Note no periodic F.I. (i.e., $[Ca^{2+}]_i$) changes in any of the graphs, due to the presence of ryanodine and thapsigargin. (B) Effect of heating on peak SLs during HSOs. IR laser irradiation (for 10 s each) was applied three times consecutively. Minimal and maximal SLs, obtained at the peak of shortening and lengthening, respectively. (C) Effect of heating on the amplitude of HSOs. Amplitude was defined as maximal SL (at the peak of lengthening) minus minimal SL (at the peak of shortening) [cf. (B)]. (D) Effect of heating on the frequency of HSOs. (E) Effect of heating on the sarcomere shortening velocity during HSOs. Velocity was calculated based on our previous study employing SL nanometry [13]. In (B), (C), (D) and (E), data obtained 1.5, 5 and 8.5 s after the onset of heating (i.e., beginning, middle and end of heating) were analyzed for 2nd and 3rd heating. Due to time delay of the appearance of HSOs [see **(A)** and Fig. S1B], data obtained 4, 6.5 and 9 s after the onset of heating (i.e., beginning, middle and end of heating) were analyzed for 1st heating. $^{*}P < 0.05$ compared with the corresponding value obtained in the beginning of heating. $^{\#}P < 0.05$ compared with the corresponding value obtained in the middle of heating. $^{\dagger}P < 0.05$ compared with the corresponding value during the 1st heating. $^{\ddagger}P < 0.05$ compared with the corresponding value during the 2nd heating.

to saw-tooth-shaped (see Fig. 2A; [19]). Likewise, a heating-induced continuous decrease in the frequency (coupled at least in part with a decrease in shortening velocity; Fig. 2E) can be explained as a result of a gradual increase in α (Table 1; [19,20]). Furthermore, the high frequency features of HSOs (i.e., ~5 Hz even at the end of the 3rd heating in non-beating myocytes; Fig. 2D), as compared to cell-SPOCs (~2 Hz at 36 °C; [13]), are likely to underlie the enhanced actomyosin activity (due to higher temperature) and the ensuing acceleration of cross-bridge detachment [13,19]. Future studies with various technologies are needed to fully uncover the effects of heating on the properties of HSOs.

One may point out that there is a qualitative difference in the findings of the present study and those of the previous studies regarding the effects of heating on the state of cardiac sarcomeres. Namely, while heating induced "oscillations" in the present study, "shortening" was observed in our previous study [9] on isolated rat

Table 1
Summary of the effects of heating on the cross-bridge attachment rate and the properties of HSOs. Our mathematical model predicts that heating increases amplitude and decreases frequency via an increase in the attachment rate constant of cross-bridges, α. Arrows, directions of change. See [19,20] for details of our model.

Parameter	Physiological meaning	+Heating		
		Parameter change	Amplitude	Frequency
α	Cross-bridge attachment rate	↑	↑	↓

ventricular myocytes. There could be two possible reasons for this apparent discrepancy: first, upon the same magnitude of heating (i.e., ΔT), the adult cardiac thin filament may be quickly shifted toward the "*on*" state from the "*off*" state, presumably via enhanced cooperative activation [22], compared with the neonatal counterpart. Second, compared with free shortening in adult cardiomyocytes, cultured myocytes are likely under auxotonic conditions [13], hence ideal for observation of SPOCs [12,13]. As mathematically simulated by us [19,20], it is considered that in our previous study with isolated adult cardiomyocytes [9], sarcomeric auto-oscillations became synchronized and accordingly exhibited shortening under isotonic conditions.

To explore the physiological relevance of HSOs, we quantified ΔT (basal temperature, 25 °C) to induce HSOs by changing the distance between the laser center and sarcomeres in beating (n = 16) and non-beating (n = 8) cardiomyocytes. As shown in Fig. 3A, HSOs were observed in sarcomeres at which ΔT was between ~12 and ~17 °C under the beating condition in 16 myocytes [4 myocytes (#13–#16) showing no HSOs]. HSOs were observed in the range of temperatures between 38.6 ± 0.3 and 41.9 ± 0.3 °C (Table 2), and three myocytes (#3, #8 and #10) exhibited contraction (without oscillations) beyond the temperature range. Following blockade of the SR functions, a slightly higher level of temperature was needed to induce HSOs (ΔT between ~15 and ~19 °C. Namely, HSOs occurred in the range of temperature between 40.5 ± 0.6 and 43.5 ± 0.3 °C (Table 2). As shown in Fig. 3B, with the exception of one myocyte (#5), sarcomeres in all myocytes were relaxed in the low temperature range, and HSOs occurred as the temperature was increased. As in beating myocytes, five myocytes (#4–#8) exhibited contraction (without oscillations) at higher temperatures (at 43–45.5 °C). In both beating and non-beating myocytes, contracted sarcomeres (due to high temperatures) were lengthened upon cessation of heating, and responded to subsequent heating by showing HSOs at appropriate temperatures. These results support the notion that HSOs occur at partial thin filament activation, when temperatures become slightly above the physiologically relevant levels (see [22] for body temperature of rat neonates, i.e., ~38 °C).

In order to investigate whether or not HSOs are induced by a smaller magnitude of ΔT, we increased the temperature from 38.0 to 41.3 °C by IR laser irradiation in myocytes (i.e., ΔT, 3.3 °C) (Fig. S3). We found that HSOs were indeed induced by a ΔT of 3.3 °C and coexisted with $[Ca^{2+}]_i$-based sarcomeric contractions (see two peaks in Fig. S3C, middle). We therefore consider that it is the absolute intracellular temperature and not ΔT that is the factor which induces HSOs, via modulation of the "*on–off*" equilibrium of the thin filament state.

Finally, we quantified the waveform properties between HSOs and cell-SPOCs (data obtained in our previous study; [13]) (Fig. S4). We found that HSOs exhibited the saw-tooth waveform, i.e., shortening time (or shortening velocity) longer (or slower) than lengthening time (or lengthening velocity), characteristic to SPOCs [12]. This finding suggests that common molecular mechanisms operate in HSOs and cell-SPOCs, as mathematically

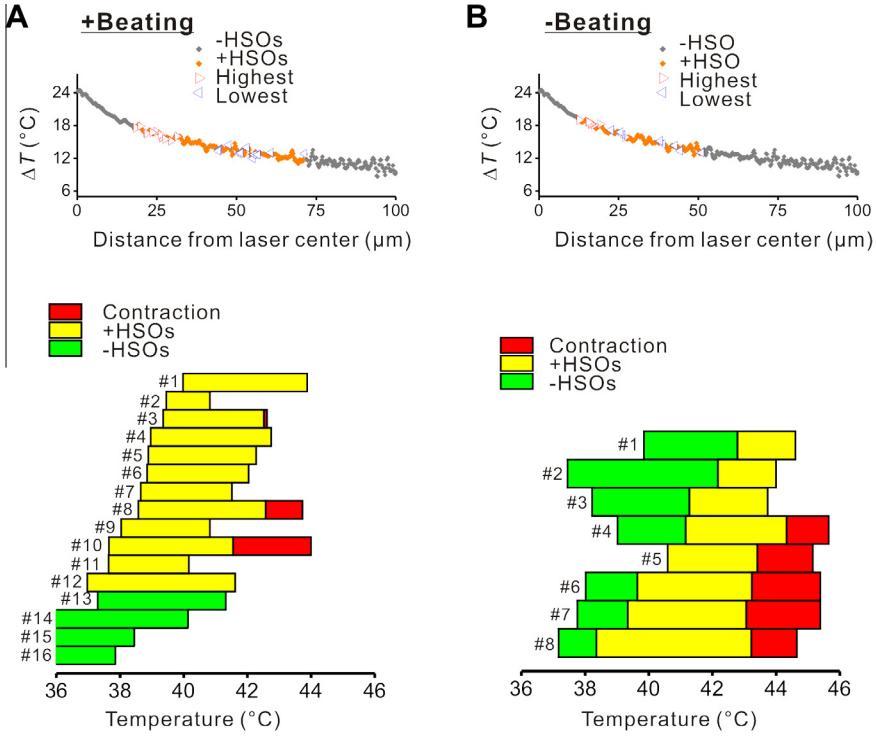

Fig. 3. Temperature dependence of the occurrence of HSOs. (A) Top: relationship between the distance from the laser center and ΔT in spontaneously beating myocytes, showing whether or not HSOs were induced by heat pulses. Myocytes were set on the microscopic system at the initial temperature of 25 °C, and the heat pulse was given for 10 s. The values of ΔT that induced HSOs are shown in orange, and those at which HSOs were not observed are shown in gray. The lowest and highest end of the temperature range for HSOs are shown in blue and red triangles, respectively. Bottom: temperature dependence of the state of sarcomeres in neonatal myocytes, i.e., spontaneous beating without HSOs (green) or spontaneous beating with HSOs (yellow), or contraction [with no oscillations (red)]. Cell numbers are indicated on left. Of the total 16 myocytes tested, four myocytes (#13–16) did not exhibit HSOs (one even at 41.5 °C). Note that the lowest temperatures at which HSOs occur in various myocytes are likely lower than those shown in this graph (Table 2). (B) Top: same as in (A) top in non-beating myocytes, showing whether or not HSOs were induced by heat pulses. As in (A), myocytes were set on the microscopic system at the initial temperature of 25 °C, and the heat pulse was given for 10 s. The values of ΔT that induced HSOs are shown in orange, and those at which HSOs were not observed are shown in gray. The lowest and highest end of the temperature range for HSOs are shown in blue and red triangles, respectively. Bottom: temperature dependence of the state of sarcomeres in neonatal myocytes, either relaxation (green), HSOs (yellow) or contraction [with no oscillations (red)]. Cell numbers are indicated on left. Of the total 8 myocytes tested, one myocyte (#5) did not exhibit relaxation, presumably because the lowest temperature at which observation started was relatively high (i.e., 40.6 °C).

Table 2
Summary of maximal and minimal temperatures for HSOs in beating and non-beating myocytes. T_{max} (T_{min}), maximal (minimal) temperature at which HSOs were observed in the experiments of Fig. 3. Note that the real T_{min} value may be even lower than the value (~38.6 °C) in this table and hence closer to the body temperature of rat neonates [22] in beating myocytes, because in all myocytes showing HSOs, the oscillations were already induced at the lowest temperatures (37–40 °C; see Fig. 3A) given. ΔT, T_{max} minus T_{min}. n, 12 and 8 for beating and non-beating myocytes, respectively.

	Beating (°C)	Non-beating (°C)
T_{min}	38.6 ± 0.3	40.5 ± 0.6*
T_{max}	41.9 ± 0.3	43.5 ± 0.3*
ΔT	3.3 ± 0.3	3.0 ± 0.4

* $P < 0.05$ compared with the corresponding values for beating myocytes.

demonstrated in our previous studies [19,20]. Detailed results and discussions are provided in the Supplementary Material.

In conclusion, we successfully induced HSOs in cultured neonatal cardiomyocytes by taking advantage of a local heating technique with IR laser irradiation. HSOs can co-exist with slower paced Ca^{2+}-dependent sarcomeric contractions. Our analyses with SL nanometry revealed that the oscillatory properties of HSOs are similar to those of SPOCs. An area of future research will be to investigate whether HSOs occur in the beating heart *in vivo* at various developmental stages in health and disease.

Author contributions

S.A.S., K.O., N.F. and S.I. designed research; S.A.S. performed research; S.A.S. analyzed data; and S.A.S., N.F. and S.I. wrote the paper.

Acknowledgments

This work was supported in part by Grants-in-Aid for Scientific Research (B) (N.F.), Scientific Research on Innovative Areas (N.F.) and Specially Promoted Research and Scientific Research (S) (S.I.) from the Ministry of Education, Culture, Sports, Science and Technology of Japan. This study was also supported in part by the Sasagawa Scientific Research Grant from The Japan Science Society (S.A.S.).

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bbrc.2014.12.077.

References

[1] W. Burrgen, A. Farrell, H. Lillywhite, Vertebrate cardiovascular systems, Handbook of Physiology, Comparative Physiology, Am. J. Physiol. Soc.,, Bethesda, MD, 1997. Sec. 13, pp. 215–308.
[2] A. Collet, T. Desaive, P.C. Dauby, One-dimensional time-dependent model of the cardiac pacemaker activity induced by the mechanoelectric feedback in a thermo-electro-mechanical background, Ann. Cardiol. Angeiol. (Paris) 61 (2012) 156–161.
[3] V. Tseeb, M. Suzuki, K. Oyama, K. Iwai, S. Ishiwata, Highly thermosensitive Ca^{2+} dynamics in a HeLa cell through IP_3 receptors, HFSP J. 3 (2009) 117–123.
[4] M.G. Shapiro, K. Homma, S. Villarreal, C.P. Richter, F. Bezanilla, Infrared light excites cells by changing their electrical capacitance, Nat. Commun. 3 (2012) 736.
[5] Q. Liu, M.J. Frerck, H.A. Holman, E.M. Jorgensen, R.D. Rabbitt, Exciting cell membranes with a blustering heat shock, Biophys. J. 106 (2014) 1570–1577.
[6] N.I. Smith et al., A femtosecond laser pacemaker for heart muscle cells, Opt. Express 16 (2008) 8604–8616.
[7] M.W. Jenkins et al., Optical pacing of the embryonic heart, Nat. Photonics 4 (2010) 623–626.
[8] M.W. Jenkins et al., Optical pacing of the adult rabbit heart, Biomed. Opt. Express 4 (2013) 1626–1635.
[9] K. Oyama et al., Microscopic heat pulses induce contraction of cardiomyocytes without calcium transients, Biochem. Biophys. Res. Commun. 417 (2012) 607–612.
[10] R.J. Solaro, H.M. Rarick, Troponin and tropomyosin: proteins that switch on and tune in the activity of cardiac myofilaments, Circ. Res. 83 (1998) 471–480.
[11] F. Kobirumaki-Shimozawa et al., Cardiac thin filament regulation and the Frank-Starling mechanism, J. Physiol. Sci. 64 (2014) 221–232.
[12] S. Ishiwata, Y. Shimamoto, N. Fukuda, Contractile system of muscle as an auto-oscillator, Prog. Biophys. Mol. Biol. 105 (2011) 187–198.
[13] S.A. Shintani et al., Sarcomere length nanometry in rat neonatal cardiomyocytes expressed with α-actinin-AcGFP in Z discs, J. Gen. Physiol. 143 (2014) 513–524.
[14] O. Zohar et al., Thermal imaging of receptor-activated heat production in single cells, Biophys. J. 74 (1998) 82–89.
[15] D.M. Bers, Excitation–Contraction Coupling and Cardiac Contractile Force, second ed., Kluwer Academic Publishers, 2001.
[16] S. Ishiwata, Studies on the F-actin.tropomyosin.troponin complex. III. Effects of troponin components and calcium ion on the binding affinity between tropomyosin and F-actin, Biochim. Biophys. Acta 534 (1978) 350–357.
[17] H. Tanaka, F. Oosawa, The effect of temperature on the interaction between F-actin and tropomyosin, Biochim. Biophys. Acta 253 (1971) 274–283.
[18] N.M. Brunet et al., Micromechanical thermal assays of Ca^{2+}-regulated thin-filament function and modulation by hypertrophic cardiomyopathy mutants of human cardiac troponin, J. Biomed. Biotechnol. 2012 (2012) 657523.
[19] K. Sato, M. Ohtaki, Y. Shimamoto, S. Ishiwata, A theory on auto-oscillation and contraction in striated muscle, Prog. Biophys. Mol. Biol. 105 (2011) 199–207.
[20] K. Sato, Y. Kuramoto, M. Ohtaki, Y. Shimamoto, S. Ishiwata, Locally and globally coupled oscillators in muscle, Phys. Rev. Lett. 111 (2013) 104–108.
[21] N. Milani-Nejad, Y. Xu, J.P. Davis, K.S. Campbell, P.M. Janssen, Effect of muscle length on cross-bridge kinetics in intact cardiac trabeculae at body temperature, J. Gen. Physiol. 141 (2013) 133–139.
[22] F.C. Howarth, M. Shafiullah, E. Adeghate, M. Ljubisavljevic, M. Jacobson, Heart rhythm disturbances in the neonatal alloxan-induced diabetic rat, Pathophysiology 18 (2010) 185–192.
[23] A.M. dos Santos, Thermal effect on Aequorea green fluorescent protein anionic and neutral chromophore forms fluorescence, J. Fluoresc. 22 (2012) 151–154.