Special Review Series Article

EXERCISE TRAINING CORRECTS CONTROL OF SPONTANEOUS CALCIUM WAVES IN HEARTS FROM MYOCARDIAL INFARCTION HEART FAILURE RATS

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ABSTRACT

Impaired cardiac control of intracellular diastolic Ca^{2+} gives rise to arrhythmias. Whereas exercise training corrects abnormal cyclic Ca^{2+} handling in heart failure, the effect on diastolic Ca^{2+} remains unstudied. Here, we studied the effect of exercise training on the generation and propagation of spontaneous diastolic Ca^{2+} waves in failing cardiomyocytes. Post-myocardial infarction heart failure was induced in Sprague-Dawley rats by coronary artery ligation. Echocardiography confirmed left ventricular infarctions of 40±5%, whereas heart failure was indicated by increased left ventricular end-diastolic pressures, decreased contraction-relaxation rates, and pathological hypertrophy. Spontaneous Ca^{2+} waves were imaged by laser linescanning confocal microscopy (488nm excitation/505-530nm emission) in 2μM Fluo-3-loaded cardiomyocytes at 37°C and extracellular Ca^{2+} of 1.2mM and 5.0mM. These studies showed that spontaneous Ca^{2+} wave frequency was higher at 5.0mM than 1.2mM extracellular Ca^{2+} in all rats, but failing cardiomyocytes generated 50% ($p<0.01$) more waves compared to sham-operated controls at Ca^{2+} 1.2mM and 5.0mM. Exercise training reduced the frequency of spontaneous waves at both 1.2mM and 5.0mM Ca^{2+} ($p<0.05$), although complete normalization was not achieved. Exercise training also increased the aborted/completed ratio of waves at 1.2mM Ca^{2+} ($p<0.01$), but not 5.0mM. Finally, we repeated these studies after inhibiting the nitric oxide synthase with L-NAME. No differential effects were found; thus, mediation did not involve the nitric oxide synthase. In conclusion, exercise training improved the cardiomyocyte control of diastolic Ca^{2+} by reducing the Ca^{2+} wave frequency and by improving the ability to abort spontaneous Ca^{2+} waves after their generation, but before cell-wide propagation.

INTRODUCTION

Heart failure (HF) is the leading cause of death, to which ventricular arrhythmias contribute ~50%, as arrhythmias lead to immediate pump failure (American Heart Association, 2010). However, no satisfactory treatment has emerged. This is partly explained by an incomplete understanding of the underlying phenomena that trigger arrhythmias, although it has become clear that impaired control of intracellular Ca^{2+} during diastole in the cardiomyocyte constitutes a major cause of arrhythmias (Venetucci et al, 2008). An important aspect of this is the increased open probability of the sarcoplasmic reticulum (SR) Ca^{2+} channel; the type 2 ryanodine receptor (RyR2). This increases the susceptibility of generating spontaneous, unsynchronized Ca^{2+} release events such as Ca^{2+} waves (MacQuaide et al, 2007) that may cause delayed afterdepolarizations (DADs), of which some reach the threshold for action potential (AP) generation that thereby trigger arrhythmias (Venetucci et al, 2008). Indeed, HF is associated with increased generation of spontaneous Ca^{2+} waves (Piacentino et al, 2003).

Corrected control of diastolic Ca^{2+} and a removal of Ca^{2+} waves in the failing cardiomyocyte has therefore major therapeutic potential for reducing the incidence of arrhythmias, but this has been difficult to achieve because Ca^{2+} or RyR2 inhibitors also interfere with excitation-contraction coupling (ECC); which on the contrary needs be sustained or increased to maintain cardiac pump function and because, albeit preliminary efforts have suggested that maneuvers modulating the RyR2 may be possible (Loughrey et al, 2007; Venetucci et al, 2006). A different approach is that of exercise training, which has little counter-indicative effects and is already recommended in HF (Conraads and Beckers, 2010). It is conceivable that exercise training has potential as a treatment option to correct dysfunctional Ca^{2+} control in diastole and thence reduce the generation and propagation of spontaneous Ca^{2+} waves in HF cardiomyocytes, because it reduces the incidence of arrhythmias (Billman et al, 2006; Holycross et al, 2007); albeit the mechanism is unknown, and does not inhibit, but rather improves ECC, Ca^{2+} cycling, and contractility (Kemi et al, 2005; Wisloff et al, 2002). However, the effect of exercise training on spontaneous Ca^{2+} waves remains unknown.

Therefore, we studied the generation, propagation, and characteristics of spontaneous Ca^{2+} waves in cardiomyocytes isolated from hearts of healthy and post-myocardial infarction (MI) HF rats that remained sedentary or underwent a chronic aerobic high-intensity exercise training program. High

intensity exercise training was chosen because the magnitude of cardiac adaptation to exercise training depends on the exercise training intensity in both healthy and HF patients and experimental animals (Haram et al, 2009; Kemi et al, 2005, 2008; Wisloff et al, 2002, 2007). Here, we report that exercise training reduces both generation and propagation of spontaneous Ca^{2+} waves in the HF cardiomyocytes. Since this effect mimics that of nitric oxide (NO) synthase (NOS) knockout on spontaneous RYR2 Ca^{2+} release (Vila Petroff et al, 2001), we further tested the possibility that the exercise training-induced correction of diastolic Ca^{2+} control was achieved through modulation of NOS-mediated NO production. A NOS-dependent mechanism is conceivable since NOS-synthesized NO modulates intracellular Ca^{2+} cycling in the cardiomyocytes (Lim et al, 2008; Tamargo et al, 2010); including aerobic fitness-related effects (Hoydal et al, 2007), and since NOS isoforms change abundance and spatial localization in HF (Damy et al, 2004; Hare and Stamler, 2005).

METHODS

Post-MI HF

3-4 month old female Sprague-Dawley with *ad libitum* access to water and a pellet rodent chow diet were used in the study. The study conforms to the *Guide for the Care and Use of Laboratory Animals* (National Institutes of Health Publication No. 85-23, revised 1996), and was approved by the Institutional Animal Research Ethics Committee.

For induction of post-MI HF, left coronary artery ligation (n=16) or sham-operation (control; n=16) was performed during thoracotomy under anesthesia provided by 1% isoflurane mixed with 30% O_2/70% N_2O, whereas Buprenorphine (0.05mg Temgesic; Reckitt and Coleman, Hull, UK) was given subcutaneously immediately and 8 hours after the thoracotomy, as previously described (Kemi et al, 2007, 2011; Wisloff et al, 2002). 7 days post-surgery, MI was confirmed by echocardiography during sedation (40mg/kg ketamine hydrochloride and 8mg/kg xylazine intraperitoneally) by tracing the endocardial circumference during 2-dimensional short axis recordings that allowed for estimation of the infarcted (non-contracting) area relative to the total circumference. At the time of sacrifice, subcutaneous anesthesia (in mL/kg: 0.33 haloperidol, 0.5 fentanyl, 0.5 midazolam, 1.3 ketamine hydrochloride, 6 H_20) was delivered, whereupon a pressure microtip catheter (size 2Fr; Millar Instruments, Houston, TX) was inserted through the right carotid artery and into the left ventricle to record pressures; 10 consecutive cardiac cycles were averaged, in order to confirm HF.

Exercise training

8 post-MI HF and 8 sham-operated rats started high-intensity exercise training 4 weeks post-surgery, by running on a motorized treadmill 1.5 hour/day, 5 days/week for 8 weeks. The exercise consisted of a 10-minute warm up, followed by 8-minute intervals at an exercise intensity of 85-90% of maximal oxygen uptake (VO_{2max}) that were interspersed by 2-minute active recovery periods at ~50% of VO_{2max} (Kemi et al, 2005; Wisloff et al, 2002). The exercise intensity was determined and controlled by measuring VO_{2max} every week by an incremental ramp test in a metabolic chamber. As controls, 8 post-MI HF and 8 sham-operated rats remained sedentary.

Cardiomyocyte experiments

Immediately after the intraventricular pressure recordings, the same hearts were excised and left ventricular cardiomyocytes remote to the infarct area originating from the viable myocardium were enzymatically isolated with a Ca^{2+}-free Krebs-Henseleit tyrode and type-2 collagenase (250 IU/mL; Worthington, Freeland, NJ), whereupon extracellular Ca^{2+} concentration ($[Ca^{2+}]$) was reintroduced stepwise to 1.2mM. After resting for ~1 hour at 37°C in a HEPES-buffered tyrode, cells were placed in a cell chamber at 37°C on an inverted microscope (Diaphot-TMD, Nikon, Tokyo, Japan) for measurements of cell length and width; 100 cells/rat. Cell volume was calculated as cell length·width·0.00759 (Kemi et al, 2011).

For measurements of spontaneous Ca^{2+} waves, cells were incubated for 20 minutes with Fluo-3/AM (2μM; Molecular Probes, Eugene, OR) in a Krebs-Henseleit tyrode with either normal (1.2mM) or high (5.0mM) extracellular $[Ca^{2+}]$ at 37°C, after which Fluo-3 was washed out before re-suspension in similar normal or high $[Ca^{2+}]$ tyrode. At least 8 cardiomyocytes from each rat and in both normal and high $[Ca^{2+}]$ tyrode were imaged at 37°C with a laser scanning confocal microscope (Axioplan 100-M LSM510, Carl Zeiss, Jena, Germany) equipped with a plan-apochromat 63x/1.4 numerical aperture oil-immersion lens. 2ms linescans along the longitudinal cardiomyocyte were recorded with a 1.0 airy unit pinhole diameter yielding an XYZ resolution of ~0.5·0.5·0.8μm, by exciting Fluo-3 with a 488nm Argon laser and capturing 505-530nm fluorescence emission with a photomultiplier tube. Care was taken to minimize the excitation laser intensity so as to minimize the photodamage to cells; which itself may trigger Ca^{2+} waves, whereas the gain of the photomultiplier tube was increased. This reduces and standardizes any generation of light-induced Ca^{2+} waves, but introduces more noise to the recorded signal, which was subsequently filtered. This yielded 512 pixel lines that were stacked and displayed as 2-dimensional images. Image processing and analysis was done by inhouse, custom-made software written in the Delphi language, whereby Ca^{2+} waves were analyzed within 10-pixel spatial bands from the record. Waves were defined by their point of origin, meaning that several waves originating simultaneously were analyzed as individual waves, whereas abortion of a wave was defined when a developed wave ceased to propagate through the cell without any interference from adjacent waves.

NOS-inhibition

From different cell batches, recording of spontaneous Ca^{2+} waves was also performed after first incubating for 30 minutes with 100μM of the highly potent NOS-inhibitor Nω-nitro-L-arginine methyl ester (L-NAME), to evaluate the contribution of cardiomyocyte NOS-derived NO.

Statistics

Data are expressed as mean±SD. One-way ANOVA with the Scheffe post hoc test evaluated differences between cells from the respective groups, with significance level $p<0.05$.

RESULTS

Myocardial performance and $\dot{V}O_{2max}$

The MI procedure resulted in infarct sizes of 40±5% of the left ventricle being ischemic and non-contracting, whereas subsequent HF was evidenced by increased left ventricular end-diastolic pressure (LVEDP) above the cut-off value of 15mmHg, reduced left ventricular peak systolic pressure (LVPSP), and reduced maximal rates of contraction ($+dP/dt_{max}$) and relaxation ($-dP/dt_{max}$), as well as 40% reduced $\dot{V}O_{2max}$ (Table 1). Moreover, post-MI HF was associated with pathological hypertrophy of the cardiomyocytes, as demonstrated by ~30% increased cell length and width and 70% increased cell volume (Table 1). Exercise training restored $\dot{V}O_{2max}$ to levels comparable to sham-operated controls and partly reversed the pathological hypertrophy, but did not restore normal hemodynamics or contraction-relaxation rates.

Spontaneous Ca^{2+} waves

Spontaneous Ca^{2+} waves were measured in resting cardiomyocytes during confocal linescanning after incubation with Fluo-3/AM (Figure 1A). 5mM extracellular [Ca^{2+}] provided a greater stimulus for generating spontaneous Ca^{2+} waves than 1.2mM in all rats, as Ca^{2+} wave frequency increased by ~100% (Figure 1B,C; $p<0.05$). More importantly, post-MI HF increased the frequency of spontaneous Ca^{2+} waves by 50% during both normal (1.2mM) and high (5mM) extracellular [Ca^{2+}] (Figure 1B,C). Exercise training partly, but not fully, reversed the frequency of Ca^{2+} wave generation toward normal levels (Figure 1B,C), whereas in sham-operated cardiomyocytes, exercise training had no effect.

A prominent feature in sedentary sham-operated and post-MI HF cardiomyocytes was that the generation of spontaneous Ca^{2+} waves resulted in waves that propagated across the complete cell. Interestingly, cardiomyocytes from both sham-operated and post-MI HF rats that had undergone exercise training showed an ability to abort 25-50% of the generated Ca^{2+} waves at 1.2mM extracellular [Ca^{2+}] (Figure 1D,E; examples of aborted waves appear also in Figure 1A in cells from sham-operated and post-MI HF exercise trained rats). Thus, exercise training introduced a ratio between aborted and complete Ca^{2+} waves in both sham-operated and post-MI HF cardiomyocytes at normal, but not high extracellular [Ca^{2+}], that was not observed in cardiomyocytes from sedentary rats. This suggests that exercise training activated an intrinsic ability to prevent propagation of a substantial proportion of the generated Ca^{2+} waves, in addition to also reducing the frequency of generation.

We also studied the characteristics of the spontaneous Ca^{2+} waves (Figure 2A), finding a small, but insignificant trend toward reduced Ca^{2+} wave amplitude in post-MI HF cardiomyocytes (p=0.1 vs. sham-operated rats); a trend that was not evident after exercise training (Figure 2B). However, post-MI HF reduced the velocity of the propagating Ca^{2+} wave by 20%, whereas exercise training increased the propagation velocity to normal levels (Figure 2C). Furthermore, whereas rise times of the propagating Ca^{2+} waves did not change with any of the interventions (Figure 2D), it was clear that post-MI HF presented with a 30% slower time to 90% decay, which was partly, but not fully normalized by exercise training (Figure 2E). In addition, exercise training in sham-operated rats also reduced the decay time, by 15%. Ca^{2+} wave morphology did not differ between normal and high extracellular [Ca^{2+}], and nor did the effects of post-MI HF and/or exercise training differ between the two different extracellular Ca^{2+} load conditions (p=0.64 1.2mM vs 5.0mM; Figure 2A). Thus, mean data obtained during extracellular [Ca^{2+}] 5.0mM are therefore not shown.

Effect of NOS-inhibition on Ca^{2+} waves

Finally, we repeated the measurements of spontaneous Ca^{2+} waves at 1.2mM extracellular [Ca^{2+}], after inhibiting NOS-derived NO production by 100μM L-NAME (Figure 3A). This maneuver had no effect on the frequency of spontaneous Ca^{2+} waves (Figure 3B) or the ratio between aborted and complete Ca^{2+} waves (Figure 3C), and nor did it modulate the effect of post-MI HF and/or exercise training (Figure 3B,C). In contrast, NOS-inhibition increased the time to 90% decay of the Ca^{2+} waves by 10-15%, but this effect was not different between groups (Figure 3D). NOS-inhibition did not affect Ca^{2+} wave rise times or velocities (data not shown). Thus, although NOS-derived NO did increase the Ca^{2+} wave decay rate, it did not contribute toward the differential effect of post-MI HF and/or exercise training on the generation, propagation, and morphology of spontaneous Ca^{2+} waves.

DISCUSSION

Here, we show that exercise training at least partly reverses the pathologic generation and propagation of spontaneous Ca^{2+} waves in failing cardiomyocytes. This is a previously unrecognized inhibitory action on intracellular Ca^{2+} handling that may hold a major potential for reducing arrhythmogenic Ca^{2+} release events that precede DADs that may cause myocardial fibrillation (MacQuaide et al, 2007; Venetucci et al, 2008). Thus, reducing generation and propagation of spontaneous Ca^{2+} waves may have an important anti-arrhythmic effect.

Spontaneous Ca^{2+} waves and DADs

Spontaneous Ca^{2+} waves are generated and propagated by opening of clusters of RyR2s on the SR membrane that lead to localized release of Ca^{2+} during diastole. This raises local intracellular [Ca^{2+}], of which the bulk is removed by the SR Ca^{2+} ATPase (SERCA2a), but also the Na^+/Ca^{2+}-exchanger (NCX). Because the NCX is electrogenic, it generates a depolarizing Na^+ current when it extrudes Ca^{2+}, which may reach threshold for triggering an AP and lead to DADs that will normally propagate across the myocardium in a regenerative manner to create ectopic beats. Thus, impaired control of diastolic Ca^{2+} by the increased open probability of the RyR2 constitutes a target that is pathologically remodeled in HF; as confirmed here and by previous studies (Piacentino et al, 2003; Venetucci et al, 2008), and which, if successfully reversed, may provide a clinical benefit by reducing the incidence of arrhythmias. In addition, reduced loss of SR Ca^{2+} during diastole may also curtail the systolic pump dysfunction in HF on a beat-to-beat basis by means of normalizing SR Ca^{2+} load (MacQuaide et al, 2007; Piacentino et al, 2003).

Effect of exercise training

The finding that exercise training almost fully reversed the spontaneous Ca^{2+} release in the failing heart may provide a treatment strategy for the arrhythmogenesis observed in HF (American Heart Association, 2010). Specifically, exercise training reduced both the generation and propagation of spontaneous Ca^{2+} waves in the post-MI HF cardiomyocytes, under both normal extracellular [Ca^{2+}] and while the cell was challenged by high extracellular Ca^{2+}, suggesting that the open probability of the RyR2 is modulated. This effect has previously only been reported in diabetic cardiomyopathy (Stolen et al, 2009). However, the finding that exercise training also increased the ability of the cardiomyocytes to prevent propagation by aborting Ca^{2+} waves is novel. These effects could be

attributable to several factors, including modulation of RyR2 gating as a consequence of altered SR Ca^{2+} load or RyR2 stability and Ca^{2+} sensitivity, as well as modulation of SR Ca^{2+} uptake by SERCA2a. The latter possibility is supported by exercise training also increasing the rate of Ca^{2+} wave decay and by exercise training restoring expression levels of SERCA2a in post-MI HF (Wisloff et al, 2002). The fact that post-MI HF also associated with reduced velocity of the propagating Ca^{2+} wave; whereas exercise training normalized this, may have been linked to architectural remodeling and reverse remodeling of the cardiomyocyte, which also occurs in these phenotypes (Kemi et al, 2011). In contrast, our experiments suggest that cardiomyocyte NOS-derived NO does not contribute toward the observed effects. This mechanism was suggested by experiments showing that NO modulates several aspects of cardiomyocyte Ca^{2+} cycling including RyR2 open probability, due to physical and chemical interactions between the RyR2 and the endothelial and neural isoforms of NOS (Lim et al, 2008; Vila Petroff et al, 2001). Moreover, the NO effect on SR Ca^{2+} release is also modulated by exercise training (Hoydal et al, 2007). However, at least under the present experimental conditions, we could not detect a role of NOS-derived NO on the generation and propagation of spontaneous Ca^{2+} waves, although inhibition of NOS did reduce the decay rate of Ca^{2+} waves, which suggests an effect on SERCA2a and is in line with previous studies (Hoydal et al, 2007).

It should be noted that species differences with regard to ECC are mainly quantitative, and not qualitative. In particular, transmembrane Ca^{2+} and Na^{+} fluxes are mainly driven by the NCX in all mammalian species, but the flux sizes may vary (Bers, 2002). This suggests that the cardioprotective effect of exercise training may apply across species including humans, albeit the magnitude of effect may differ. This however remains to be studied.

CONCLUSIONS

This study suggests that exercise training is a potent modulator of the generation and propagation of spontaneous intracellular Ca^{2+} waves in the cardiomyocyte, and that it through this route at least partly may improve the control of diastolic Ca^{2+} in HF in ways that may reduce the incidence of cardiac arrhythmias. Specifically, exercise training reduced the frequency of spontaneous Ca^{2+} waves and increased the ability of the cardiomyocyte to abort a substantial fraction of the generated Ca^{2+} waves before propagating across the cell. This action of exercise training is previously unrecognized, and may confer an important therapeutic benefit.

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FIGURE LEGENDS

Figure 1. A: (i) Configuration of laser scanning confocal imaging of cardiomyocytes with the line depicting positioning of the linescan. (ii) Example traces of linescan images showing spontaneous Ca^{2+} waves in sedentary (SED) and exercise trained (TR) sham-operated (SHAM) and post-myocardial infarction heart failure (HF) rats at 1.2mM and 5.0mM extracellular Ca^{2+} concentrations ($[Ca^{2+}]$). B: Frequency of Ca^{2+} waves at 1.2mM extracellular $[Ca^{2+}]$. C: Frequency of Ca^{2+} waves at 5.0mM extracellular $[Ca^{2+}]$. D: Ratio between aborted and complete Ca^{2+} waves at 1.2mM extracellular $[Ca^{2+}]$. E: Ratio between aborted and complete Ca^{2+} waves at 5.0mM extracellular $[Ca^{2+}]$. Difference SHAM vs MI: A, $p<0.01$; a, $p<0.05$. Difference HF SED vs HF TR: B, $p<0.01$; b, $p<0.05$. Difference SHAM SED vs SHAM TR: C; $p<0.01$. Data are mean±SD with 8 animals/group (8-12 cells/animal).

Figure 2. A: Example profiles of Fluo-3/AM fluorescence showing spontaneous Ca^{2+} waves in sedentary (SED) and exercise trained (TR) sham-operated (SHAM) and post-myocardial infarction heart failure (HF) rats at 1.2mM and 5.0mM extracellular Ca^{2+} concentrations ($[Ca^{2+}]$). B: Amplitude of Ca^{2+} waves at 1.2mM extracellular $[Ca^{2+}]$. C: Velocity of Ca^{2+} waves at 1.2mM extracellular $[Ca^{2+}]$. D: 10-90% rise time of Ca^{2+} waves at 1.2mM extracellular $[Ca^{2+}]$. E: Time to 90% decay of Ca^{2+} waves at 1.2mM extracellular $[Ca^{2+}]$. Difference SHAM vs MI: A, $p<0.01$; a, $p<0.05$. Difference HF SED vs HF TR: b, $p<0.05$. Note that since no differences occurred between 1.2mM and 5.0mM extracellular $[Ca^{2+}]$, only average data from 1.2mM $[Ca^{2+}]$ is presented. Data are mean±SD with 8 animals/group (8-12 cells/animal).

Figure 3. A: Example traces of linescan images showing spontaneous Ca^{2+} waves in sedentary (SED) and exercise trained (TR) sham-operated (SHAM) and post-myocardial infarction heart failure (HF) rats at 1.2mM extracellular Ca^{2+} concentration ($[Ca^{2+}]$) after incubation with 100µM L-NAME. B: Frequency of Ca^{2+} waves at 1.2mM extracellular $[Ca^{2+}]$ after incubation with L-NAME. C: Ratio between aborted and complete Ca^{2+} waves at 1.2mM extracellular $[Ca^{2+}]$ after incubation with L-NAME. D: Time to 90% decay of Ca^{2+} waves at 1.2mM extracellular $[Ca^{2+}]$ after incubation with L-NAME; note that difference to vehicle is illustrated by the dotted line. Difference SHAM vs MI: A, $p<0.01$; a, $p<0.05$. Difference HF SED vs HF TR: b, $p<0.05$. Difference SHAM SED vs SHAM TR: c;

$p<0.05$. Difference vehicle vs L-NAME: *, $p<0.05$. Data are mean±SD with 8 animals/group (8-12 cells/animal).

Table 1. Myocardial hemodynamics, exercise capacity, and cardiomyocyte morphology.

	SHAM SED	SHAM TR	HF SED	HF TR
LVEDP (mmHg)	4±3	4±3	25±4A	22±3A
LVPSP (mmHg)	110±12	115±14	77±10A	78±9A
$+dP/dt_{max}$ ($mmHg \cdot ms^{-1}$)	9±2	9±1	3±2A	4±2A
$-dP/dt_{max}$ ($mmHg \cdot ms^{-1}$)	8±2	8±1	3±1A	4±1A
VO_{2max} ($mL \cdot kg^{-1} \cdot min^{-1}$)	45±4	62±5C	28±3A	47±3B
Cell Length (μm)	101±5	111±4C	132±7A	116±5AB
Cell Width (μm)	23±2	24±1	31±3A	27±2AB
Cell Volume (pL)	18±2	21±1C	31±4A	24±3AB

SHAM: sham-operated control; SED: sedentary; TR: exercise training; HF: heart failure; LVEDP: left ventricle end-diastolic pressure; LVPSP: left ventricle peak systolic pressure; $+dP/dt_{max}$: peak rate of left ventricle pressure rise (contraction); $-dP/dt_{max}$: peak rate of left ventricle pressure fall (relaxation); VO_{2max}: maximal oxygen uptake (exercise capacity); A: $p<0.01$ difference SHAM vs HF; B: $p<0.01$ difference HF SED vs HF TR; C: $p<0.01$ difference SHAM SED vs SHAM TR. Data are mean±SD with 8 animals/group, and 100 cells/rat constituting the cell morphology of each rat.

Figure 1

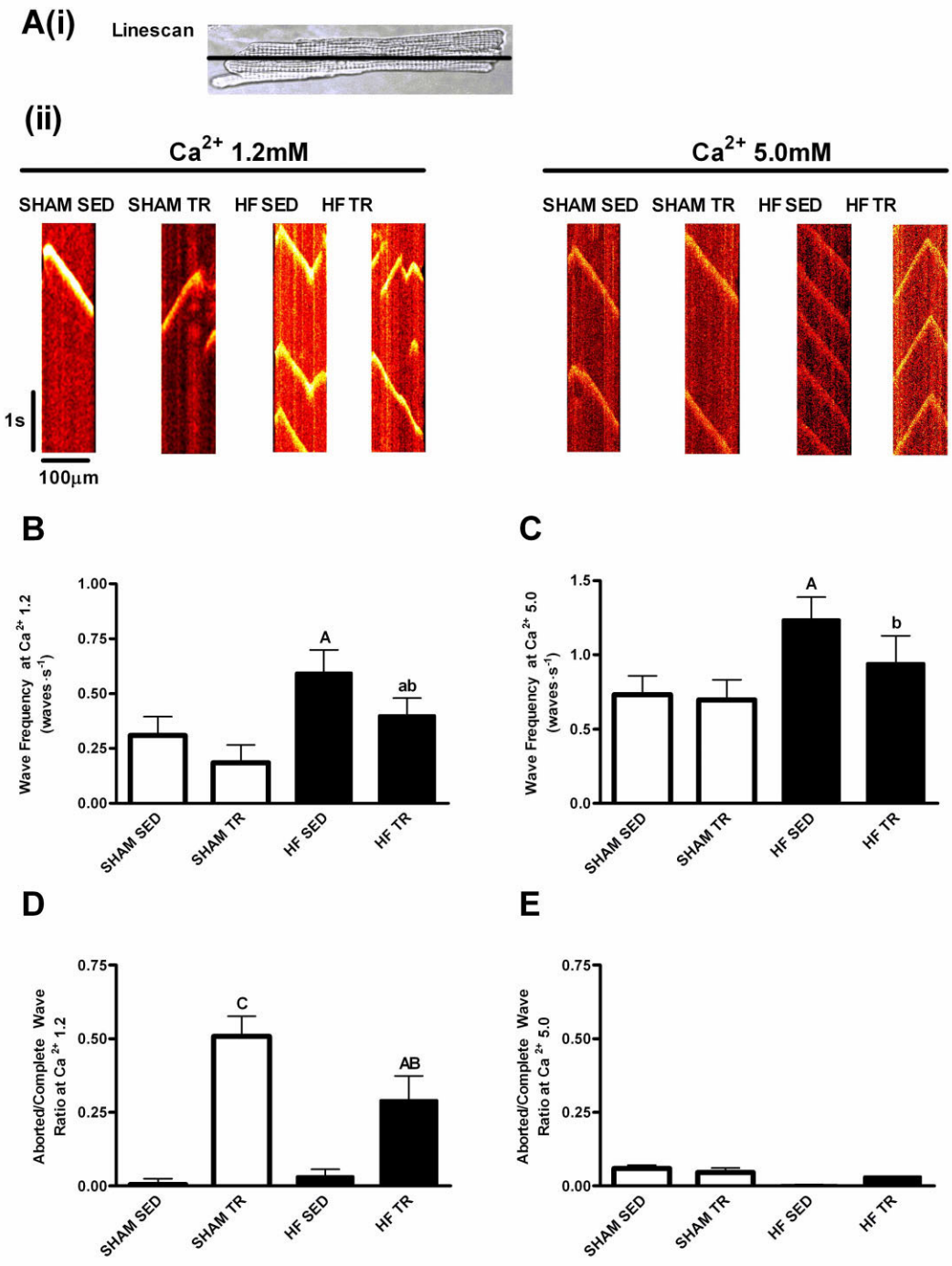

Figure 2

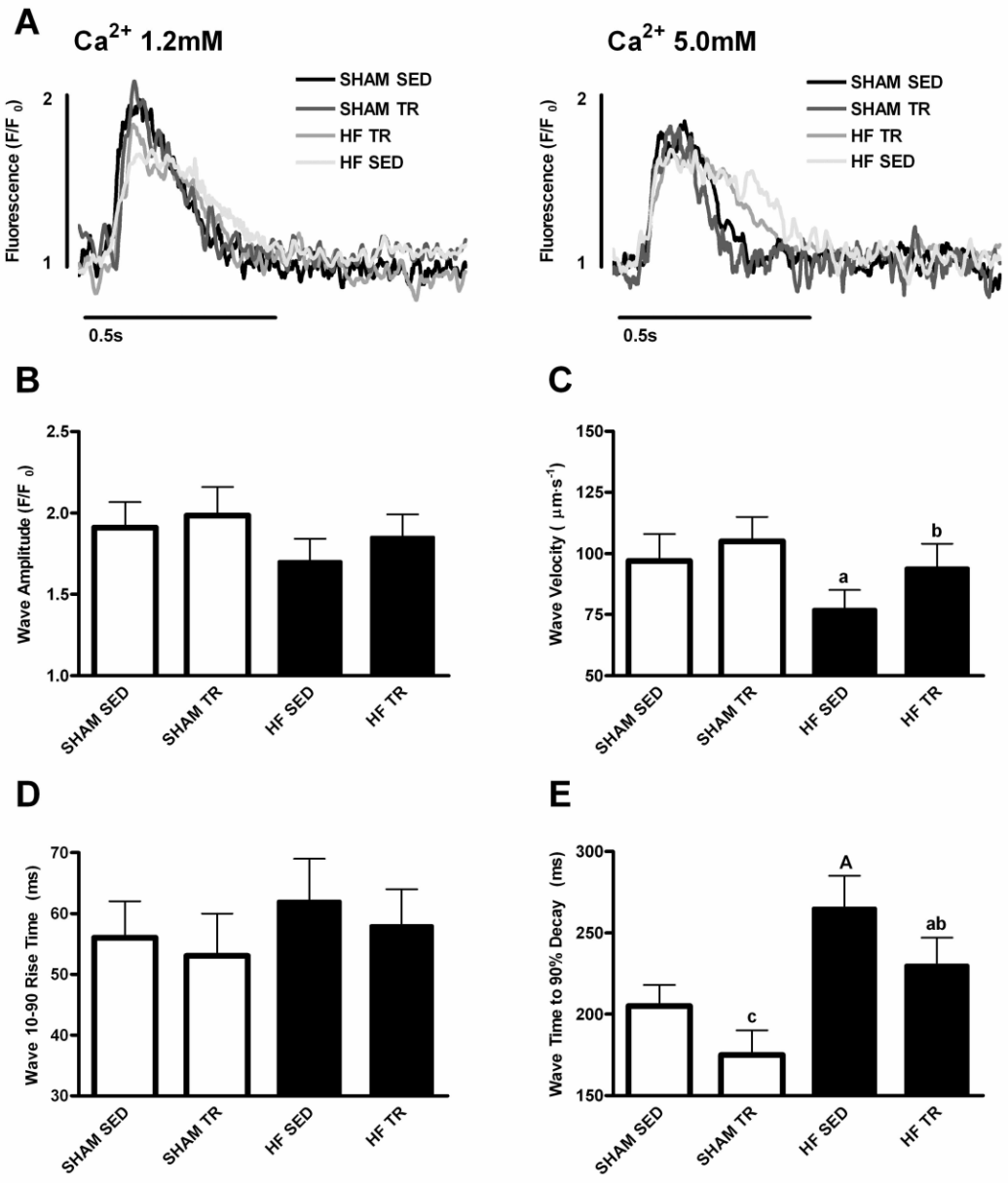

Figure 3