Comparison of cardiac excitation–contraction coupling in isolated ventricular myocytes between rat and mouse

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Abstract

Transgenic animals offer many advantages for physiological study. The mouse is the most extensively utilized mammalian model for gene modification. Isolated ventricular myocytes are pivotal for assessment of cardiac function by allowing direct cellular and environmental manipulation without interference from compensatory mechanisms that may exist in vivo. This study was designed to compare the basic excitation–contraction coupling properties of mouse and rat ventricular myocytes. Cardiac myocytes were isolated from age- and gender-matched mice (FVB and C57BL/6) and rats (Sprague–Dawley (SD) and Wistar). Mechanical and intracellular Ca properties were measured with an IonOptix SoftEdge system, including peak shortening (PS), time-to-PS (TPS), time-to-90% relengthening (TR⁹⁰), maximal velocity of shortening and relengthening (±dL/dt), and intracellular Ca²⁺ fura-2 fluorescence intensity and decay rate (τ). Resting cell length was variable among the different species or strains. PS from FVB group was significantly higher than the SD group. TPS and TR⁹⁰ were significantly shorter in mice. ±dL/dt was similar among all groups whereas −dL/dt was significantly faster in the C57BL/6 group compared to the rat groups. Resting intracellular Ca²⁺ was lower in mice than in rats, and Ca²⁺-induced Ca²⁺ release was variable among the four groups. Intracellular Ca²⁺ decay was slower in Wistar compared to all other groups. The myocytes from C57BL/6 did not respond to increases in extracellular Ca²⁺. Myocytes from the FVB group exhibited a lesser reduction in PS in response to elevated stimulus frequency. These data suggest that inherent differences between strains or species should be taken into consideration when comparing results from these different animal models.

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1. Introduction

The mouse has been the major focus of transgenic techniques and has become the most extensively used mammalian model for genetic modification studies, including those involved in assessing cardiac function. However, few reports have been published dealing with cardiac excitation–contraction (E–C) coupling at the level of isolated mouse myocytes. The rat species has been used extensively for myocardial function study, largely due to well-established myocyte isolation and manipulation techniques (Hintz et al., 2001; Wold et al., 2001). Improved mouse myocyte isolation techniques have made the mouse increasingly useful in cardiac physiological studies, especially in conjunction with transgenic techniques.
(Lewis et al., 2000; Duan et al., 2002). For this reason it has become increasingly essential to characterize the intrinsic properties of mouse myocytes for direct comparison with rat studies of similar nature.

Wistar and Sprague–Dawley (SD) rat strains have been used extensively in cardiac contractile studies for years (Hintz et al., 2001; Wold et al., 2001). Although the mouse has been utilized infrequently for myocardial study in transgenic models and myocyte studies, the FVB and C57BL/6 mouse strains have recently been used (Lewis et al., 2000; White et al., 2001; Duan et al., 2002). Significant differences in hypertrophic signaling have been indicated in cardiac myocytes from neonatal mouse cell cultures in direct comparison with rat cultures (Deng et al., 2000). Mice myocardium have also been found to exhibit increased sensitivity to extracellular Ca$^{2+}$ compared to the rat (Brooks and Conrad, 1999).

This study compares the fundamental characteristics of cardiac E–C coupling in isolated ventricular myocytes from two strains of rats and two strains of mice. Our data reveal substantial differences in cardiac E–C coupling between mouse and rat myocytes.

2. Materials and methods

2.1. Experimental animals and cell isolation procedures

All animal procedures described in this study were approved by the Institutional Animal Care and Use Committee at the University of North Dakota School of Medicine. Male rats (Wistar and SD) and mice (FVB and C57BL/6) were purchased at 4 weeks of age from Harlan SD (Indianapolis, IN) and the National Cancer Institute (Bethesda, MD), respectively. They were housed in separate cages in temperature-controlled rooms under a 12-h light/12-h dark cycle and allowed access to standard lab chow and tap water ad libitum. The animals were sacrificed at 7 months of age for ventricular myocyte isolation according to previously described methods (Ren and Wold, 2001; Duan et al., 2002). In brief, after ketamine/xylazine sedation (5:3, 1.32 mg/kg, i.p.) of the animals, the hearts were removed and perfused with Krebs-Henseleit bicarbonate (KHB) buffer containing (in mM): 118 NaCl, 4.7 KCl, 1.2 MgSO$_4$, 1.2 KH$_2$PO$_4$, 25 NaHCO$_3$, 10 HEPES and 11.1 glucose, with 5% CO$_2$–95% O$_2$. The rat hearts were subsequently digested with 223 U/ml collagenase II (Worthington Biochemical Corp., Freehold, NJ) for 15–20 min whereas the mouse hearts were perfused with 0.9 mg/ml collagenase D (Boehringer Mannheim Biochemicals, Indianapolis, IN) for the same duration. After perfusion, the ventricles were removed, minced in KHB buffer, and filtered to remove membrane debris. Extracellular Ca$^{2+}$ was slowly increased to 1.25 mM. The nature and quality of the myocyte isolation procedure is the single most important factor in the integrity of myocytes recovered from enzymatic trauma and the success in their mechanical assessment. A good heart isolation often yielded 70–80% (rats) and 50–60% (mice) viable rod-shaped ventricular myocytes with clear sacromere striations. The cell survival and viability appear to be comparable between rats and mice within the first 4–8 h of isolation, during which time the mechanical assessment was conducted. Myocytes with obvious sarcolemmal blebs or spontaneous contractions were not used.

2.2. Cell shortening/relengthening

The mechanical properties of ventricular myocytes from both species were assessed using a video-based edge-detection system (IonOptix, Milton, MA) as described previously (Ren, 2000). In brief, cells were placed in a chamber mounted on the stage of an inverted microscope (Olympus IX-70) and superfused (~2 ml/min at 30 °C) with a buffer containing (in mM): 131 NaCl, 4 KCl, 1 CaCl$_2$, 1 MgCl$_2$, 10 mM glucose, 10 HEPES, at pH 7.4. The cells were then field stimulated to contract at a frequency of 0.5 Hz and the myocyte being studied was rapidly scanned with a camera at 120 Hz to ensure good recording fidelity. Changes in cell length (CL) during shortening and relengthening were captured and converted to a digital signal before being analyzed with IonOptix software. Cell shortening and relengthening were assessed using the following indices: peak shortening (PS), time-to-PS (TPS) and time-to-90% relengthening (TR$_{90}$), and maximal velocities of shortening and relengthening ($\pm$ dL/dr). Steady state contraction of myocytes was achieved before data was recorded.

2.3. Intracellular fluorescence measurement

Myocytes were loaded with fura-2/AM (0.5 µM) for 10 min at 30 °C, and fluorescence
measurements were recorded with a dual-excitation fluorescence photomultiplier system (IonOptix) as previously described (Ren, 2000). Myocytes were imaged through an Olympus IX-70 Fluor 40× oil objective. While being stimulated to contract at 0.5 Hz, cells were exposed to light emitted by a 75-W lamp and passed through either a 360 or 380 nm filter (bandwidths were ±15 nm). Fluorescence emissions were detected between 480 and 520 nm by a photomultiplier tube after first illuminating the cells at 360 nm for 0.5 s then at 380 nm for the duration of the recording protocol (333 Hz sampling rate). The 360 excitation scan was repeated at the end of the protocol and qualitative changes in intracellular Ca²⁺ concentration ([Ca²⁺]) were inferred from the ratio of the fluorescence intensity at the two wavelengths.

2.4. Statistical analyses

For each experimental series, data are presented as mean ± S.E.M. Statistical significance (P < 0.05) for each variable was estimated by analysis of variance or t-test, where appropriate (SYSTAT, Inc., Evanston, IL). A Dunnett’s post hoc analysis was also implemented when necessary.

3. Results

3.1. General features of mouse and rat

The mice exhibited significantly higher heart, liver and kidney weight to body weight ratios compared to the rats. There were no differences between the two strains of the same species in any of the biometric indices tested, with the only exception being that the liver/body weight ratio was significantly smaller in the C57BL/6 strain compared to the FVB group (Table 1).

3.2. Myocyte shortening and relengthening properties between rat and mouse myocytes

All cardiac myocytes used in this study were randomly chosen within the first 4–8 h of isolation. The average resting CL was significantly different between the two rat strains (Wistar and SD) as well as between the two mouse strains (FVB and C57BL/6). Myocytes from the FVB group were also significantly shorter than those from the Wistar group (Fig. 1A). The PS amplitude normalized to CL was essentially similar among all the groups tested, except that PS was significantly greater in the FVB group than the SD group (Fig. 1B). Both mouse species exhibited significantly shorter TPS and TR₉₀ compared to the rat groups (Fig. 1C and D). TPS and TR₉₀ were significantly different between the groups within the same species (with the exception of TR₉₀ between the two mouse groups). The inter-species/strain difference in TPS was not associated with a significant variation in maximal velocity of shortening (+dL/dt) (Fig. 1E). However, the shortened TR₉₀ in C57BL/6 myocytes compared to the rat groups was accompanied with an enhanced maximal velocity of relengthening (−dL/dt) (Fig. 1F).

3.3. Properties of intracellular Ca²⁺ transients in myocytes from rat and mouse hearts

In order to determine if the differences in cardiac myocyte mechanical properties between different species or strains was due to discrepant intracellular Ca²⁺ homeostasis, we used the membrane

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Wistar (n=11)</th>
<th>SD (n=11)</th>
<th>FVB (n=12)</th>
<th>C57BL/6 (n=11)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight (g)</td>
<td>4.01 ± 0.89</td>
<td>4.14 ± 1.12</td>
<td>2.3 ± 0.8</td>
<td>2.56 ± 0.9</td>
</tr>
<tr>
<td>Heart weight (g)</td>
<td>1.81 ± 0.11</td>
<td>1.70 ± 0.05</td>
<td>0.20 ± 0.01</td>
<td>0.19 ± 0.02</td>
</tr>
<tr>
<td>Heart wt./body wt. (mg/g)</td>
<td>4.54 ± 0.29</td>
<td>4.11 ± 0.12</td>
<td>8.25 ± 0.23</td>
<td>7.09 ± 0.92</td>
</tr>
<tr>
<td>Liver weight (g)</td>
<td>13.2 ± 0.4</td>
<td>13.4 ± 0.8</td>
<td>1.26 ± 0.05</td>
<td>1.20 ± 0.13</td>
</tr>
<tr>
<td>Liver wt./body wt. (mg/g)</td>
<td>33.0 ± 1.1</td>
<td>32.1 ± 1.9</td>
<td>52.8 ± 1.0</td>
<td>43.1 ± 4.57</td>
</tr>
<tr>
<td>Kidney weight (g)</td>
<td>2.76 ± 0.07</td>
<td>2.70 ± 0.11</td>
<td>0.37 ± 0.02</td>
<td>0.38 ± 0.04</td>
</tr>
<tr>
<td>Kidney wt./body wt. (mg/g)</td>
<td>6.89 ± 0.18</td>
<td>6.52 ± 0.33</td>
<td>15.7 ± 0.4</td>
<td>13.7 ± 1.5</td>
</tr>
</tbody>
</table>

wt, weight; SD, Sprague–Dawley. Animal numbers are provided in parentheses (n); mean ± S.E.M.

* P < 0.05 vs. other strain of same species.
permeant form of fura-2 to evaluate intracellular Ca\(^{2+}\) transients in myocytes from all four groups. The time course of the fluorescence signal decay was described by a single exponential Chebyshev equation, and the time constant (τ) was used as a measure of the rate of intracellular Ca\(^{2+}\) clearing. The fluorescence measurements revealed that the resting intracellular Ca\(^{2+}\) level was generally lower in myocytes from mice than from rats. The resting intracellular Ca\(^{2+}\) level was also significantly different between the Wistar and SD groups (Fig. 2A). The electrically stimulated Ca\(^{2+}\)-induced Ca\(^{2+}\) release (Δ[Ca\(^{2+}\)]\(_{\text{rest}}\)=peak[Ca\(^{2+}\)]−resting[Ca\(^{2+}\)]\(_{\text{rest}}\)) and intracellular Ca\(^{2+}\) transient...
Fig. 2. Intracellular Ca transient properties in left ventricular myocytes freshly isolated from 7-month-old male rat (Wistar and SD) and mouse (FVB and C57BL/6) hearts. Graphs depicted are (A) resting intracellular Ca levels ([Ca^{2+}]); (B) electrically stimulated Ca-induced Ca release ([Ca^{2+}]); and (C) intracellular Ca transient decay constant (τ). All myocytes were stimulated to contract at 0.5 Hz at 30°C. Rod-shaped striated myocytes were randomly chosen for study. Mean±S.E.M.; n = 87–101 cells from a total of 11–12 animals per group. (a) P < 0.05 vs. Wistar group; (b) P < 0.05 vs. SD group; (c) P < 0.05 vs. FVB group; (d) P < 0.05 vs. C57BL/6 group.

3.4. Effect of extracellular Ca^{2+} on myocyte shortening in myocytes from rat and mouse hearts

To evaluate the myofilament responsiveness to Ca^{2+}, the effect of extracellular Ca^{2+} on myocyte shortening was examined and is shown in Fig. 3. Responses are expressed as a percentage of that obtained in the contractile medium with the lowest Ca^{2+} concentration (0.5 mM). Increases in extracellular Ca^{2+} concentration up to 3 mM resulted in a positive staircase in myocyte shortening response in the Wistar, SD and FVB groups. Interestingly, the myocytes from the C57BL/6 group did not exhibit a positive staircase in response to increased extracellular Ca^{2+} but displayed a significant negative response, suggesting potential distinct myofilament Ca^{2+} sensitivity mechanisms in myocytes from C57BL/6 mice compared to the other three groups. However, potential strain-related differences in cardiac decay rate were significantly higher and slower, respectively, in Wistar group compared to all other groups. The Δ[Ca^{2+}]i was also significantly different between the two mouse groups (Fig. 2B and C). These results reveal potential differences in intracellular Ca^{2+} handling and clearing mechanisms among the different species of rat and mouse examined in this study. Myocyte shortening was also recorded from fura-2 loaded cells but was used for qualitative comparisons only, in order to avoid potential effects on contraction from intracellular Ca^{2+} buffering by fura-2.
Fig. 4. PS amplitude of ventricular myocytes freshly isolated from 7-month-old male rat (Wistar and SD) and mouse (FVB and C57BL/6) hearts. The cells were stimulated to contract at different stimulus frequencies (0.1–5.0 Hz). Each point represents PS amplitude normalized to that of 0.1 Hz. Mean±S.E.M.; cell numbers are provided in the parentheses.

**P* < 0.05 vs. all other groups, **P* < 0.05 vs. rat strains.

responsiveness to the enzymatic digestion cannot be ruled out at this time.

3.5. Effect of stimulation frequency on myocyte shortening between rat and mouse myocytes

Rodent hearts normally contract at very high frequencies (> 300 beats/min at 37 °C), whereas our baseline stimulus was 0.5 Hz (30 beats/min). To investigate possible derangement of cardiac E–C coupling at higher frequencies, we incrementally increased the stimulating frequency to 5 Hz (300 beat/min) and recorded the steady-state PS. Cells were initially stimulated to contract at 0.5 Hz for 5 min to ensure steady-state before commencing the frequency study. All of the recordings were normalized to the PS obtained at 0.1 Hz of the same myocyte. Fig. 4 shows a negative staircase in PS with increasing stimulating frequency that is somewhat comparable among all four groups, with the exception that FVB myocytes display a smaller reduction in PS with a moderate increase in stimulus frequency (0.5 and 1 Hz). This data suggests that the intracellular Ca²⁺ storage and release may be more efficient in FVB hearts under moderate stress. Changes in the stimulating frequency from 0.1 to 5 Hz did not affect the pattern of distribution in TPS and TR₉₀ in myocytes from rat and mouse hearts (data not shown).

4. Discussion

Transgenic mouse models have become increasingly useful in cardiac studies over the past few years. The recent use of these models in the mechanical evaluation of isolated cardiac myocytes broadens clinical as well as experimental significance of the transgenic mouse in cardiovascular research. The study presented here is the first attempt to characterize and compare the cardiac E–C coupling properties between rats and mice. Our results indicate that certain discrepancies do exist in cardiac E–C coupling between species and between strains that warrant consideration when choosing an experimental animal model or when comparing cardiac contractile data obtained from different animals. The major mechanical differences between rat and mouse myocytes observed in our study included elevated PS, shortened duration of contraction and relaxation, and elevated velocity of relengthening associated with different intracellular Ca²⁺ homeostasis in FVB, C57BL/6 mice, or both. In addition, myocytes from the C57BL/6 mice exhibited reduced responsiveness to increased extracellular Ca²⁺, whereas myocytes from FVB mice displayed more efficient intracellular Ca²⁺ storage and release under moderate stress. These findings indicate that species differences should be taken into account when using transgenic mouse models for myocardial contractile study.

Mechanical function is an important component of evaluating cardiac performance. Overt mechanical abnormalities may be observed in animal models of diabetes, hypertension and obesity, and are often characterized as prolonged duration of contraction and relaxation (Ren et al., 1999; Ren and Bode, 2000; Ren and Wold, 2001). The findings from our current study suggest that the most prominent difference in cardiac function between mouse and rat myocytes is the shortened duration of contraction and relaxation (TPS and TR₉₀) in mouse myocytes compared to rat myocytes. This may be largely due to the fact that the fast-type V₁ isoform is the predominant form of myosin heavy chain in mouse hearts in contrast to the predominant slow-type V₃ isoform in rat hearts (Ng et al., 1991; Brooks et al., 1987). Since diabetes and hypertension themselves may directly lead to a switch in myosin heavy chain isoform
(Dillmann, 1989), caution should be taken when comparing cardiac mechanical properties between rats and mice under these morbidities.

Altered mechanical function is often due to changes in intracellular Ca\(^{2+}\) handling. It has been shown that the properties of intracellular Ca\(^{2+}\) transients are directly responsible for the mechanical function of single cardiac myocytes on a beat-to-beat basis (Ren and Davidoff, 1997; Ren and Bode, 2000). Our current study revealed that electrically stimulated Ca\(^{2+}\)-induced Ca\(^{2+}\) release are higher in Wistar myocytes compared to those of mice, indicating that a larger intracellular pool of Ca\(^{2+}\) may be available for recruitment upon excitation. The intracellular Ca\(^{2+}\) transient decay is also significantly slower, associated with a higher resting Ca\(^{2+}\) level, in myocytes from the Wistar group compared to those from all other groups, suggesting that the ability to remove/clear intracellular Ca\(^{2+}\) after contraction is not as efficient in the Wistar myocytes. This claim is strengthened by our previous findings that the Wistar rat is a better model than the SD for displaying diabetic cardiomyopathy when Streptozotocin is used to induce experimental diabetes (Ren et al., unpublished data). Further studies are warranted to examine the expression of sarco(endoplasmic reticulum Ca\(^{2+}\)-ATPase (SERCA) and Na\(^+\)/Ca\(^{2+}\) exchanger (NCX) among various rodent models. Reduced function of SERCA and NCX has been shown to account for slowed Ca\(^{2+}\) clearing and elevated resting intracellular Ca\(^{2+}\) levels in diabetes (Russ et al., 1991; Chattou et al., 1999).

In conclusion, we demonstrated certain species- and strain-related differences in cardiac E–C coupling in ventricular myocytes. These results suggest that as far as cardiac E–C coupling is concerned, the mouse is not merely a small rat. Therefore, caution must be taken when comparing mechanical properties from different rodent models.

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**References**


