Influence of age and run training on cardiac Na\(^+\)/Ca\(^{2+}\) exchange

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Mace, Lisa C., Bradley M. Palmer, David A. Brown, Korinne N. Jew, Joshua M. Lynch, Jason M. Glunt, Todd A. Parsons, Joseph Y. Cheung, and Russell L. Moore. Influence of age and run training on cardiac Na\(^+\)/Ca\(^{2+}\) exchange. J Appl Physiol 95: 1994–2003, 2003. First published July 25, 2003; 10.1152/japplphysiol.00551.2003.—Effects of age and training on myocardial Na\(^+\)/Ca\(^{2+}\) exchange were examined in young sedentary (YS; 14–15 mo), aged sedentary (AS; 27–31 mo), and aged trained (AT; 8- to 11-wk treadmill run training) male Fischer Brown Norway rats. Whole heart performance and isolated cardiocyte Na\(^+\)/Ca\(^{2+}\) exchange characteristics were measured. At the whole heart level, a small but significant slowing of late isovolumic left ventricular (LV) relaxation, which may be indicative of altered Na\(^+\)/Ca\(^{2+}\) exchange activity, was seen in hearts from AS rats. This subtle impairment in relaxation was not observed in hearts from AT rats. At the single-cardiocyte level, late action potential duration was prolonged, resting membrane potential was more positive, and overshoot potential was greater in cardiocytes from AS rats than from YS rats (P < 0.05). Training did not influence any of these age-related action potential characteristics. In electrically paced cardiocytes, neither shortening nor intracellular Ca\(^{2+}\) concentration ([Ca\(^{2+}\)]\(_i\)) dynamics was influenced by age or training. Similarly, neither age nor training influenced the rate of [Ca\(^{2+}\)]\(_i\) clearance via forward (Na\(^+\)/Ca\(^{2+}\)) exchange after caffeine-induced Ca\(^{2+}\) release from the sarcoplasmic reticulum or cardiocytes with fluorescence microscopy were used to evaluate the ability of Na\(^+\)/Ca\(^{2+}\) exchange to alter cytosolic ([Ca\(^{2+}\)]\(_c\)) ([Ca\(^{2+}\)]\(_i\)) under conditions where membrane potential (V\(_m\)) and internal and external Na\(^+\) and Ca\(^{2+}\) could be controlled, we observed age-associated increases in forward Na\(^+\)/Ca\(^{2+}\) exchange-mediated [Ca\(^{2+}\)]\(_i\) clearance (P < 0.05) that were not influenced by training. The age-related increase in forward Na\(^+\)/Ca\(^{2+}\) exchange activity provides an hypothetical explanation for the late action potential prolongation observed in this study.

cafeine; NCX1; Fisher Brown Norway rat; treadmill; heart; cardiocyte; calcium; sodium

THE CARDIAC SARCOLEMMALE Na\(^+\)/Ca\(^{2+}\) exchanger is centrally involved in the beat-to-beat regulation of cellular Ca\(^{2+}\) content and cardiac contractile force. Within a single contraction-relaxation cycle, electrogenic Na\(^+\)/Ca\(^{2+}\) exchange is thought to significantly influence action potential configuration in the late repolarization phase (49). In addition, Na\(^+\)/Ca\(^{2+}\) exchange has been shown to be involved in the later Ca\(^{2+}\) clearance phase of the intracellular Ca\(^{2+}\) concentration ([Ca\(^{2+}\)]\(_i\)) transient in isolated ventricular cardiocytes (44), suggesting possible involvement in the later phase of myocardial relaxation. Because advanced aging elicits the prolongation of both myocardial relaxation and the duration of the action potential (5, 17, 18, 25, 37, 38, 45), it is tempting to speculate that advanced age elicits alterations in myocardial Na\(^+\)/Ca\(^{2+}\) exchange activity. An age-dependent reduction in forward (Na\(^+\)/Ca\(^{2+}\)) Na\(^+\)/Ca\(^{2+}\) exchange activity could contribute to slowed intracellular Ca\(^{2+}\) clearance and mechanical relaxation, whereas an age-dependent increase in Na\(^+\)/Ca\(^{2+}\) exchange could generate a depolarizing current and contribute to a prolongation in late action potential duration.

From studies of enriched sarcolemmal vesicles or muscle strips isolated from rat, Na\(^+\)/Ca\(^{2+}\) exchange activity has been reported to be decreased (13), increased (9), or unaffected (1) in aged myocardium. The basis for this lack of consensus is not known, but it may be related to methodological differences in membrane vesicle preparation or experimental models. Specifically, factors that influence Na\(^+\)/Ca\(^{2+}\) exchange activity, including membrane potential (V\(_m\)) and transsarcolemmal Ca\(^{2+}\) and Na\(^+\) gradients, or other cytosolic modulators of Na\(^+\)/Ca\(^{2+}\) exchange activity may not be functionally operational or observable in studies of membrane vesicles and muscle strips.

Consequently, we sought to determine the effects of advanced age on Na\(^+\)/Ca\(^{2+}\) exchange activity in left ventricular (LV) cardiocytes isolated from the rat. By using isolated cardiocytes, Na\(^+\)/Ca\(^{2+}\) exchange activity could be assessed under conditions in which V\(_m\) and/or transsarcolemmal Ca\(^{2+}\) and Na\(^+\) gradients could be controlled and in a setting in which the most basic levels of cellular organization were still intact. Whole heart performance and action potential characteristics were assessed to determine whether a correlation exists between age-related changes in Na\(^+\)/Ca\(^{2+}\) exchange activity and myocardial relaxation or repolarization. In addition, exercise training has been shown...
to improve mechanical relaxation in aged myocardium (12, 34, 37, 39), whereas it does not appear to reverse age-related action potential prolongation (12). Our working hypothesis was that Na\(^+/\)Ca\(^{2+}\) exchange activity would be reduced in ventricular cardiocytes isolated from aged rats compared with their young sedentary counterparts and that endurance training would normalize (increase) Na\(^+/\)Ca\(^{2+}\) exchange. These alterations would mirror the age- and training-related alterations in myocardial relaxation. An alternative to our working hypothesis is that advanced age would bring about an increase in myocardial Na\(^+/\)Ca\(^{2+}\) exchange activity that would be unaffected by endurance training and that these alterations would be associated with changes in the duration of the later phase of action potential repolarization. Contrary to our working hypothesis, the results of our studies indicate that action potential repolarization was incrementally increased each day for 2 wk (5 days/wk). As presented in Fig. 1, the speed, duration, or both were incrementally increased each day for 2 wk (5 days/wk). After this introductory period, the daily training protocol consisted of a 5-min warm-up at 14 m/min, followed by 40 min at 17.5 m/min, 5 days/wk, for at least 8 and no more than 11 wk.

**METHODS**

**Animal model.** Young sedentary (YS; 14–15 mo), aged sedentary (AS; 27–31 mo), and aged trained (AT) male Fisher Brown Norway (PBN) rats were used in this study. This rat strain was used for its relatively well-known and nonpathological response to advancing age (22, 43). All animals were maintained on a 12:12-h light-dark cycle and provided food and water ad libitum. Animal protocols received prior approval from the Institutional Animal Care and Use Committee at the University of Colorado at Boulder and were conducted under the guidelines accepted by the American Physiological Society. The run training protocol was conducted over a minimum of 10 wk on motorized treadmills kept at a 10% grade. AT rats were initially introduced and familiarized to the treadmill with a single 5-min walk (<14 m/min). As presented in Fig. 1, the speed, duration, or both were incrementally increased each day for 2 wk (5 days/wk). After this introductory period, the daily training protocol consisted of a 5-min warm-up at 14 m/min, followed by 40 min at 17.5 m/min, 5 days/wk, for at least 8 and no more than 11 wk.

**Cardiocyte pacing and caffeine contraction experiment.** A Nikon Diaphot microscope fitted with a ×40 oil-immersion objective was used for all experiments. [Ca\(^{2+}\)]_i dynamics were measured with the method and fluor excitation filter pairing of Palmer et al. (32) and Palmer and Moore (31). Data were analyzed with a computer equipped with I onWizard 5.0 software (IonOptix, Milton, MA).

In our experiments, steady-state cardiocyte shortening dynamics and fluorescence ratio (R) data were recorded from cardiocytes isolated from 9 YS (39 cells), 11 AS (31 cells), and 11 AT (46 cells) rats after 7 min of electrical pacing. Only R data were recorded during caffeine-induced contractions in cardiocytes isolated from 9 YS (32 cells), 9 AS (26 cells), and 10 AT (39 cells) rats. We used a protocol previously established by our laboratory (29, 30), except that fura 5F (Molecular Probes, Eugene, OR) was used as the fluorescent Ca\(^{2+}\)
indicator for its high selectivity for Ca\(^{2+}\) and wide dynamic range \((K_a = 400 \text{ nM})\). An example of an R transient recorded during electrical stimulation and caffeine exposure and the R characteristics measured are illustrated in Fig. 2.

Recorded R transients for both electrically paced and caffeine-exposed data were corrected for background fluorescence on a cell-by-cell basis and analyzed with IonOptix software as previously described \((29, 32)\). It should be noted that the nomenclature for certain fluorescence metrics is similar among the pacing, caffeine contraction, and voltage-clamp protocols. Therefore, the subscript \((p)\) is used to specify the measurements derived from paced cardiocytes, the subscript \((c)\) for the caffeine contraction measurements, and the subscript \((v)\) for the voltage-clamp measurements (see Voltage clamp experiment). The key R characteristics assessed during pacing were baseline (diastolic) R \([R_{\text{rest}(p)}]\), time-to-peak R \([\text{TTP}(p)]\), R difference \([R - R_{\text{rest}(p)}]\), designated \(R_{\text{v}(p)}\), and the exponential rate constant, \([k_{\text{fall}(p)}]\). The R characteristics analyzed for the caffeine contraction experiment were baseline (diastolic) R immediately before caffeine exposure \([R_{\text{rest}(c)}]\), R difference \([R - R_{\text{rest}(c)}]\), designated \(R_{\text{v}(c)}\), R plateau occurring during caffeine exposure before the reintroduction of extracellular Na\(^+\) \([R_{\text{eq}(c)}]\), and the exponential rate constants \(k_{\text{eq}(c)}\) and \(k_{\text{flux}(c)}\). All rate constants were determined by nonlinear, least-squares fitting of exponential functions to the recorded R transient \((15, 29, 30)\).

Voltage-clamp experiment. Whole cell \(V_m\) was controlled with an Axopatch 200B amplifier (Axon Instruments, Foster City, CA) and recorded onto a computer with pCLAMP 8.1 software (Axon Instruments). Cytosolic [Ca\(^{2+}\)]/[(Ca\(^{2+}\)]_c) dynamics were measured as described in Cardiocyte pacing and caffeine contraction experiment, with the exception that fura 2 free acid (Molecular Probes) was used as the [Ca\(^{2+}\)]_c indicator.

[Ca\(^{2+}\)]_c data were recorded under voltage-clamp control in cardiocytes isolated from 11 YS (28 cells), 15 AS (25 cells), and 13 AT (21 cells) rats with a modification of the protocol established by Barcenas-Ruiz et al. \((2)\). Na\(^+\)/Ca\(^{2+}\) exchange was isolated from other cellular ion transport mechanisms by pharmacological blockade and chemical substitution with a bathing solution containing \((\text{in mM})\) 136.3 NaCl, 1 MgCl\(_2\), 2.4 CaCl\(_2\), 7.5 CsCl, 7.5 tetraethylammonium (TEA), 2.5 glucose, 0.5 pyruvate, and (in \(\mu M\)) 25 ryanodine, 3.75 thapsigargin, and 7.45 verapamil, pH 7.4 with NaOH. Verapamil was used to block L-type Ca\(^{2+}\) channel activity, thapsigargin to block sarcoplasmic reticular (SR) Ca\(^{2+}\) uptake, ryanodine to block SR Ca\(^{2+}\) release, and TEA and CsCl to block K\(^+\) currents. Cardiocytes were internally dialyzed for 5 min with a pipette \((1.3–2.5 \text{ M})\) filling solution containing the free acid form of the calcium indicator fura 2 \((20 \text{ M})\) and \((\text{in mM})\) 5 NaCl, 130 CsCl, 2 MgCl\(_2\), 10 HEPES, and 2 Na\(_2\)ATP, pH 7.2 with CsOH. Experiments were performed at 24 ± 1°C.

Our voltage-clamp protocol was designed to force Na\(^+\)/Ca\(^{2+}\) exchange to work in both the forward \((\text{Na}_\text{out}/\text{Ca}_\text{in})\) and reverse \((\text{Na}_\text{in}/\text{Ca}_\text{out})\) directions. Briefly, 2,500-ms voltage steps \((\text{to } -40, 0, \text{ and } +40 \text{ mV})\) were initiated from a holding potential of \(-80 \text{ mV}\) (see Fig. 3). At the end of each voltage step, the \(V_m\) was returned to \(-80 \text{ mV}\) and the next sweep was initiated after a 2-min interval. The three voltage steps \((\text{to } -40, 0, \text{ and } +40 \text{ mV})\) were selected to stimulate reverse Na\(^+\)/Ca\(^{2+}\) exchange and intracellular Ca\(^{2+}\) loading. Repolarization to the holding potential \((-80 \text{ mV})\) was achieved by 10.22 ± 0.33.6 on April 18, 2017 http://jap.physiology.org/ Downloaded from

Fig. 2. An example of fluorescence ratio (R) transients recorded during electrically induced and caffeine-induced contractions. The R transients occurring in response to electrical pacing were recorded after a contractile steady state had been achieved. A caffeine contracture was initiated by the rapid application of a superfusate containing neither Na\(^+\) nor Ca\(^{2+}\) as indicated \((\text{between } 6 \text{ and } 11 \text{ s})\). An Na\(^+\)-rich superfusate was then applied, and the rate of Na\(^+\)-dependent Ca\(^{2+}\) efflux, presumed to be due to forward Na\(^+\)/Ca\(^{2+}\) exchange, was characterized by the rate constant \(k_{\text{efflux}}\). Other characteristics of intracellular Ca\(^{2+}\) concentration \([(\text{Ca}\(^{2+}\)]_c)\) dynamics that were analyzed include resting R during pacing \([R_{\text{rest}(p)}]\), peak R value during pacing \([R - R_{\text{rest}(p)}]\), designated \(R_{\text{v}(p)}\), the exponential rate constant describing R transient return to \(R_{\text{rest}(p)}\) \([k_{\text{fall}(p)}]\), resting R immediately before caffeine exposure \([R_{\text{eq}(c)}]\), peak R value elicited by caffeine exposure \([R_{\text{rest}(c)}]\), the rate constant describing the transition from the initial caffeine-induced R transient to \(R_{\text{eq}(c)}\) \([k_{\text{eq}(c)}]\), and the plateau R value occurring just before exposure of the cell to extracellular Na\(^+\) \([R_{\text{eq}(c)}]\).
with unequal variances are more conservative, and per the principles described by Williams et al. (46), the use of the latter significance minimizes the propensity to commit a type II interpretive error. All data in Figures 5, 6, and 8, and Tables 1–5 are presented as means ± SE.

RESULTS

Morphology, Western blot, and LV contractile function. Both age and training significantly increased cardiocyte length, width, and area (Table 1). Neither training nor age individually affected tibial length or adrenal weights. Body and spleen weights significantly increased with age, but training either prevented or reversed the age-related increases, respectively. Plantaris citrate synthase activity levels were significantly increased in AT compared with both AS and YS (P < 0.05), verifying peripheral training adaptations as previously reported (27).

To evaluate NCX1 expression, septal and LV free wall tissue samples from YS (n = 12), AS (n = 11), and AT (n = 8) hearts were homogenized and used for Western blot protein analysis with monoclonal antibody R3F1 that recognizes a single band (NCX1) at an apparent molecular mass of 160 kDa as shown in Fig. 4. Because previous work showed calsequestrin protein (CLSQ) levels to be uninfluenced by advanced age in the heart (48), CLSQ expression was used as an internal standard (48). After phosphorimager quantification, no between-group or regional differences were found in NCX1 and CLSQ expression or in NCX1-to-CLSQ ratios (P > 0.05).

Isovolumic left heart experiment. Advanced age modestly impaired late LV relaxation in paced, isovolumic hearts (Table 2). The time required for hearts to achieve 75% (T75) and 90% (T90) maximal relaxation was slightly but significantly prolonged in hearts from AS rats compared with hearts from the YS animals (P < 0.05). Training improved late relaxation to the extent that T90 was significantly shorter in AT than AS hearts (P < 0.05).

Cardiocyte pacing and caffeine contraction experiment. Neither age nor training significantly affected cardiocyte shortening characteristics (data not shown). Steady-state ratiometric fluorescence characteristics of the electrically paced cardiocytes are presented in Table 3. Rrest(p), Rdiff(p), and Rfall(p) were not affected by advanced age or run training. TTP(p) was slower in AT

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Table 1. Effects of age and run training on animal and cardiocyte morphology and muscle biochemistry

<table>
<thead>
<tr>
<th></th>
<th>YS</th>
<th>AS</th>
<th>AT</th>
</tr>
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<tbody>
<tr>
<td>Cell length, µm</td>
<td>128 ± 1 (740)</td>
<td>133 ± 1* (795)</td>
<td>138 ± 1† (404)</td>
</tr>
<tr>
<td>Cell width, µm</td>
<td>37.8 ± 0.4 (740)</td>
<td>39.8 ± 0.4* (796)</td>
<td>41.5 ± 0.6† (403)</td>
</tr>
<tr>
<td>Cell area, µm²</td>
<td>3977 ± 40 (740)</td>
<td>4272 ± 44* (794)</td>
<td>4665 ± 73† (404)</td>
</tr>
<tr>
<td>Body weight, g</td>
<td>502 ± 8 (17)</td>
<td>565 ± 7* (27)</td>
<td>484 ± 6* (26)</td>
</tr>
<tr>
<td>Tibial length, mm</td>
<td>43.0 ± 0.3 (17)</td>
<td>43.4 ± 0.3 (23)</td>
<td>43.4 ± 0.2 (25)</td>
</tr>
<tr>
<td>Left adrenal, mg</td>
<td>18 ± 2 (17)</td>
<td>23 ± 2 (26)</td>
<td>25 ± 2* (23)</td>
</tr>
<tr>
<td>Right adrenal, mg</td>
<td>17 ± 2 (16)</td>
<td>19 ± 1 (25)</td>
<td>20 ± 1 (22)</td>
</tr>
<tr>
<td>Spleen, mg</td>
<td>690 ± 10 (17)</td>
<td>870 ± 15* (27)</td>
<td>708 ± 15† (25)</td>
</tr>
</tbody>
</table>

Values are means ± SE for no. of cardiocytes or animals in parentheses. YS, young sedentary rats; AS, aged sedentary rats; AT, aged trained rats. *P < 0.05 compared with YS; †P < 0.05 compared with AS.
cardiocytes than in YS cardiocytes ($P < 0.05$). No other differences in fluorescence dynamics were observed between groups.

The R dynamics of caffeine-stimulated cardiocytes are shown in Table 4. Similar to results from the paced cardiocytes, $R_{\text{rest(c)}}$ was not affected by advanced age or training. Values for $R_{\text{diff(c)}}$, $k_{\text{efflux}}$, and $R_{\text{equil}}$ were also not influenced by age or training.

Voltage-clamp experiment. In these experiments, we examined $V_m$-dependent changes in $[\text{Ca}^{2+}]_c$ that were produced by forward and reverse $\text{Na}^+/\text{Ca}^{2+}$ exchange (see Fig. 3). Time-to-peak $[\text{Ca}^{2+}]_c$ ($\text{TTP}_{(v)}$), defined as the time elapsed between the onset of membrane depolarization and the apex of the $[\text{Ca}^{2+}]_c$ transient, was not influenced by advanced age or training ($\text{TTP}_{(v)}$ values for the voltage step to $+40 \text{ mV}$: $\text{YS} = 688 \pm 80 \text{ ms}$, $\text{AS} = 731 \pm 130 \text{ ms}$, and $\text{AT} = 601 \pm 60 \text{ ms}$; $P > 0.05$). It is relevant to note that the long $\text{TTP}_{(v)}$ values observed in this study (ranging from $-400$ to $1,200 \text{ ms}$) provide evidence that SR $\text{Ca}^{2+}$ release was successfully eliminated by pharmacological blockade (see METHODS) and that the voltage-dependent increase in $[\text{Ca}^{2+}]_c$ was due to reverse $\text{Na}^+/\text{Ca}^{2+}$ exchange. Neither the rate of $[\text{Ca}^{2+}]_c$ rise ($k_{\text{rise}}$) values expressed in s$^{-1}$ for the voltage step to $+40 \text{ mV}$: $\text{YS} = 5.2 \pm 0.7$, $\text{AS} = 5.1 \pm 0.9$, and $\text{AT} = 5.4 \pm 0.6$; $P > 0.05$) nor peak $[\text{Ca}^{2+}]_c$ difference (peak difference values expressed in nM for the voltage step to $+40 \text{ mV}$: $\text{YS} = 32.6 \pm 5.3$, $\text{AS} = 24.9 \pm 3.7$, and $\text{AT} = 27.7 \pm 5.5$; $P > 0.05$) were affected by age or training.

In each voltage-dependent $[\text{Ca}^{2+}]_c$ transient, a $[\text{Ca}^{2+}]_c$ plateau was evident during the last third of each voltage step (beginning $\sim$1.5 s into the voltage step), and this plateau value was designated $\text{CaPlat}$ (see Fig. 3A). We found direct age-associated reductions in $\text{CaPlat}$, with the AS group exhibiting significantly lower $\text{CaPlat}$ values than the YS group ($P < 0.05$; Fig. 5).

Advanced age was associated with an increase in the repolarization-induced rate of $[\text{Ca}^{2+}]_c$ fall ($k_{\text{fall(vol)}}$) in patch-clamped cardiocytes compared with YS cardiocytes ($P < 0.01$; Fig. 6). This finding is indicative of an age-associated increase in forward $\text{Na}^+/\text{Ca}^{2+}$ exchange activity ([Ca$^{2+}]_c$ clearance).

Action potential experiment. $\text{V}_{\text{rest}}$ was more positive in cardiocytes from both aged groups compared with YS cardiocytes ($P < 0.05$; Table 5). $V_{\text{max}}$ and action potential amplitude were significantly greater in AS cardiocytes relative to YS cardiocytes ($P < 0.05$). Action potential duration was not different between groups until the later phase of recovery and then only in response to advanced age, not training. The time required to return to 75% and 90% of $V_{\text{rest}}$ from $V_{\text{max}}$, $\text{T}_{75}$ and $\text{T}_{90}$, was significantly increased with age ($P < 0.05$).

**Table 3. Effects of age and training on fluorescence dynamics during electrical pacing**

<table>
<thead>
<tr>
<th></th>
<th>YS ($n = 37$)</th>
<th>AS ($n = 31$)</th>
<th>AT ($n = 48$)</th>
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<tbody>
<tr>
<td>$R_{\text{rest(p)}}$</td>
<td>$0.65 \pm 0.01$</td>
<td>$0.65 \pm 0.02$</td>
<td>$0.60 \pm 0.02$</td>
</tr>
<tr>
<td>$R_{\text{diff(p)}}$</td>
<td>$0.21 \pm 0.02$</td>
<td>$0.19 \pm 0.01$</td>
<td>$0.22 \pm 0.02$</td>
</tr>
<tr>
<td>$\text{TTP}_{(p), \text{ms}}$</td>
<td>$67 \pm 2$</td>
<td>$71 \pm 2$</td>
<td>$75 \pm 2^*$</td>
</tr>
<tr>
<td>$k_{\text{fall(p), s}^{-1}}$</td>
<td>$6.5 \pm 0.3$</td>
<td>$6.5 \pm 0.5$</td>
<td>$5.6 \pm 0.3$</td>
</tr>
</tbody>
</table>

Values are means $\pm$ SE; $n =$ no. of cardiocytes. Ratiometric fluorescence (R) characteristics analyzed: $R_{\text{rest(p)}}$, baseline (diastolic) R; $R_{\text{diff(p)}}$, peak R $- R_{\text{rest(p)}}$; $\text{TTP}_{(p)}$, time to peak R; $k_{\text{fall(p)}}$, exponential rate of return to baseline from peak R. $^* P < 0.05$ compared with YS.

**Table 4. Effects of age and training on fluorescence dynamics during caffeine contraction**

<table>
<thead>
<tr>
<th></th>
<th>YS ($n = 32$)</th>
<th>AS ($n = 26$)</th>
<th>AT ($n = 39$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$R_{\text{rest(c)}}$</td>
<td>$0.67 \pm 0.02$</td>
<td>$0.61 \pm 0.02$</td>
<td>$0.65 \pm 0.02$</td>
</tr>
<tr>
<td>$R_{\text{diff(c)}}$</td>
<td>$0.31 \pm 0.02$</td>
<td>$0.26 \pm 0.02$</td>
<td>$0.31 \pm 0.02$</td>
</tr>
<tr>
<td>$k_{\text{equil}}, \text{s}^{-1}$</td>
<td>$1.25 \pm 0.08$</td>
<td>$1.30 \pm 0.08$</td>
<td>$1.28 \pm 0.09$</td>
</tr>
<tr>
<td>$k_{\text{efflux}}, \text{s}^{-1}$</td>
<td>$0.74 \pm 0.05$</td>
<td>$0.82 \pm 0.05$</td>
<td>$0.89 \pm 0.05$</td>
</tr>
<tr>
<td>$R_{\text{equil}}$</td>
<td>$0.08 \pm 0.01$</td>
<td>$0.09 \pm 0.02$</td>
<td>$0.08 \pm 0.01$</td>
</tr>
</tbody>
</table>

Values are means $\pm$ SE; $n =$ no. of cardiocytes. Ratiometric fluorescence (R) characteristics analyzed: $R_{\text{rest(c)}}$, baseline (diastolic) R measured immediately before caffeine exposure; $R_{\text{diff(c)}}$, peak R during caffeine exposure $- R_{\text{rest(c)}}$; $k_{\text{equil}}$, rate constant describing the transition of from the initial caffeine-induced R transient to $R_{\text{equil}}$; $k_{\text{efflux}}$, rate constant describing Na$^+$-dependent Ca$^{2+}$ efflux, presumed to be due to forward Na$^+/\text{Ca}^{2+}$ exchange; $R_{\text{equil}}$ plateau R value occurring just before exposure of the cell to extracellular Na$^+$. 

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0.05) and can be seen in representative action potential tracings in Fig. 7. This age-associated prolongation was also evident in the AT cardiocytes, albeit to a lesser extent (P < 0.10).

**DISCUSSION**

Slowed myocardial relaxation and action potential prolongation are commonly observed consequences of advanced aging (5, 16, 18, 23, 35, 37, 38, 44, 45). These two findings in the aging literature give rise to two mutually exclusive hypotheses for how advanced age might influence Na+/Ca2+ exchange function. First, because of its involvement in cardiocyte Ca2+ regulation, it is conceivable that decreases in Na+/Ca2+ exchange activity could contribute to age-related changes in myocardial relaxation. Alternatively, because of its electrogentic properties it is also possible that increases in Na+/Ca2+ exchange could be responsible for prolonged late action potential duration. Advanced age has been reported to decrease (13), increase (9), or not influence (1) Na+/Ca2+ exchange function in studies of isolated membrane vesicles and cardiac muscle strip preparations. Consequently, we directly examined the effects of aging and training on Na+/Ca2+ exchange function in intact ventricular cardiocytes and on NCX1 expression in ventricular myocardium.

We found no age-, training-, or region-related differences in NCX1 expression in our studies. At a fundamental level, these findings are significant in that they would indicate that neither advanced age (AS) nor training superimposed on advanced age (AT) elicits alterations in myocardial Na+/Ca2+ exchange activity via alterations in the amount of NCX1 expressed in the heart. On the other hand, because NCX1 activity can

<table>
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<th>Table 5. Effects of age and run training on characteristics of cardiocyte action potentials</th>
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<tr>
<td>Vrest, mV</td>
</tr>
<tr>
<td>Vmax, mV</td>
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<tr>
<td>AP amplitude, mV</td>
</tr>
<tr>
<td>T10, ms</td>
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<td>T25, ms</td>
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<tr>
<td>T50, ms</td>
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<tr>
<td>T75, ms</td>
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<td>T90, ms</td>
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Values are means ± SE; n = no. of action potentials (AP). Vrest, resting membrane potential; Vmax, maximal membrane potential; AP amplitude, Vmax - Vrest; T10, T25, T50, T75, and T90, time required for membrane potential to return to 10%, 25%, 50%, 75%, and 90% of Vrest from Vmax, respectively. *P < 0.05 compared with YS; †P < 0.10 compared with YS.
be modulated by a myriad of factors, our Western blot data cannot be interpreted to mean that myocardial Na\(^+/\)Ca\(^{2+}\) exchange activity is uninfluenced by advanced age or training. Consequently, we used two different approaches to examine the operation of Na\(^+/\)Ca\(^{2+}\) exchange in intact cardiocytes isolated from rat ventricular myocardium.

One approach involved the use of isolated cardiocytes that were electrically paced and then subjected to rapid solution changes that were designed to isolate and allow for the characterization of Na\(^+/\)Ca\(^{2+}\) exchange-mediated Ca\(^{2+}\) clearance from the cell. Rapid solution switching strategies were used to isolate and characterize effects of advanced age and training on Na\(^+\)-dependent Ca\(^{2+}\) removal from ventricular cardiocytes. We used this approach previously (29) to demonstrate that in mature adult rats a program of high-intensity endurance training elicits a reduction in the rate of cellular Ca\(^{2+}\) clearance via forward Na\(^+/\)Ca\(^{2+}\) exchange, as reflected by the rate constant \(k_{\text{efflux}}\) describing Na\(^+/\)Ca\(^{2+}\) exchange-mediated Ca\(^{2+}\) clearance from the cell. In the present study, neither advanced age nor training influenced \(k_{\text{efflux}}\). These negative results are subject to two different interpretations. First, if taken at face value, these findings indicate that in intact cardiocytes possessing a native intracellular milieu, neither advanced age nor training noticeably alters the contribution of Na\(^+/\)Ca\(^{2+}\) exchange to sarcoplasmic Ca\(^{2+}\) clearance. The lack of influence of training on \(k_{\text{efflux}}\) in the present study may have been due to the fact that the aged animals were not able to train as intensely as the younger animals that were used in the earlier studies (29). Alternatively, it is possible that small age- or training-induced alterations in intrinsic Na\(^+/\)Ca\(^{2+}\) exchange function may have been obscured in our experimental context. It is well known that Na\(^+/\)Ca\(^{2+}\) exchange function is subject to control by \(V_m\) and the magnitude of transsarclemmal [Na\(^+\)] and [Ca\(^{2+}\)] gradients. In our caffeine contraction experiments, we could tightly control the composition of the extracellular milieu, but we did not have control of or quantitative information about \(V_m\) and transsarclemmal [Na\(^+\)] and [Ca\(^{2+}\)] gradients across our three experimental groups. Therefore, we conducted experiments to assess Na\(^+/\)Ca\(^{2+}\) exchange function under conditions in which these key control parameters could be standardized.

Our whole cell voltage-clamp experiments allowed us to examine Na\(^+/\)Ca\(^{2+}\) exchange-mediated changes in cardiocyte [Ca\(^{2+}\)] under conditions in which \(V_m\) was carefully controlled, cells were internally dialyzed with solutions of known composition, and changes in [Ca\(^{2+}\)]\(_i\) could be directly monitored. Under these tightly controlled conditions, the voltage-induced elevation in [Ca\(^{2+}\)]\(_i\) was greater in YS than AS cardiocytes, perhaps because of age-induced diminution in reverse (Na\(^+/\)out/ Ca\(^{2+}\)\(_i\)) Na\(^+/\)Ca\(^{2+}\) exchange activity late in the voltage step (Fig. 5). It is generally thought that under most physiological conditions, the regulatory importance of cellular Ca\(^{2+}\) influx via reverse Na\(^+/\)Ca\(^{2+}\) exchange is quite minor (4), and it is difficult to ascertain what the physiological significance of our finding might be.

More importantly, on hyperpolarization of Ca\(^{2+}\)-loaded cardiocytes, we did find evidence of an age-induced increase in forward Na\(^+/\)Ca\(^{2+}\) exchange activity that was not influenced by exercise training (see Fig. 6). This finding is significant for several reasons. Forward (Na\(^+/\)out/Ca\(^{2+}\)\(_i\)) Na\(^+/\)Ca\(^{2+}\) exchange is central to overall cardiocyte Ca\(^{2+}\) homeostasis because it is the primary cellular Ca\(^{2+}\) efflux mechanism involved in quantitatively offsetting the Ca\(^{2+}\) influx that occurs during normal excitation-contraction coupling on a beat-to-beat basis. Efflux of Ca\(^{2+}\) via forward Na\(^+/\)Ca\(^{2+}\) exchange has been implicated in late myocardial relaxation (3, 49), and, owing to the electrogenic nature of Na\(^+/\)Ca\(^{2+}\) exchange, it has also been shown to influence late action potential repolarization (49). The age-associated acceleration in forward Na\(^+/\)Ca\(^{2+}\) exchange-mediated Ca\(^{2+}\) clearance that we observed is not consistent with and cannot explain the age-associated prolongation in late LV relaxation, and subsequent normalization with training, that we reported in this study (Table 2). If late LV relaxation were significantly influenced by forward Na\(^+/\)Ca\(^{2+}\) exchange, we would have expected to see an age-associated decrease, rather than an increase, in this type of exchange activity in isolated cardiocytes. Our forward Na\(^+/\)Ca\(^{2+}\) exchange data are, however, sufficient to explain the late action potential prolongation that we observed in cardiocytes from aged rats.

Because forward Na\(^+/\)Ca\(^{2+}\) exchange produces a net inward current (3Na\(^+\)/1Ca\(^{2+}\)\(_o\)), an increase in forward activity may influence action potential characteristics via membrane depolarization. In this study, we found that with advanced age, \(V_{\text{rest}}\) was slightly but significantly more positive, \(V_{\text{max}}\) was significantly more positive, and late action potential repolarization was slowed (Table 5). In different rat models of advanced aging, early and late action potential prolongation and pronounced \(V_{\text{max}}\) have also been observed (5, 12, 23, 44, 45), but none of these earlier studies addressed the ionic basis for late action potential prolongation (see below). We are aware of only one other study in which the effects of training on action potential characteristics were examined in myocardium isolated from aged rats. Whereas we found no evidence that training significantly altered any of the age-related changes in the cardiac action potential, Gwathmey et al. (12) reported that training tended to normalize the age-induced increase in \(V_{\text{max}}\) while further prolonging action potential duration. That our results do not exactly corroborate the findings of Gwathmey et al. (12) is not too surprising given the rat strain (FBN vs. Fischer 344) and training protocol (e.g., running speed, grade, and duration) differences between the two studies. What is intriguing in our study is that the age-related prolongations in late action potential duration that we observed were associated with the increases that we observed in forward Na\(^+/\)Ca\(^{2+}\) exchange. Although the precise ionic events responsible for the age-dependent prolongation of the late repolarization phase of the rat
action potential have not been previously identified, it has been suggested that a Ca\(^{2+}\)-dependent inward current (24), perhaps a Na\(^{+}/\)Ca\(^{2+}\) exchange current (44), may be involved.

The idea that Na\(^{+}/\)Ca\(^{2+}\) exchange may contribute to prolonged action potential duration is supported by our work in that age-related increases in forward Na\(^{+}/\)Ca\(^{2+}\) exchange were correlated with the changes in late action potential duration that we observed (see Fig. 8). Although the correlation certainly does not prove causality, it does provide a circumstantial case for the involvement of Na\(^{+}/\)Ca\(^{2+}\) exchange in this age-dependent phenomenon. This is physiologically significant because it is known that hearts exhibiting prolonged action potential duration often display proarrhythmic phenomena (3).

In this study, the age-related increase in ventricular cardiocyte cell dimension and the slowing of LV relaxation corroborate the central cardiac effects of advanced aging described by others using different rat strains (8, 19, 23, 45). In addition, when endurance exercise training was superimposed on our model of aging, we observed further increases in cardiocyte dimension and an improvement in the lusitropic function of the heart isolated from aged animals. These types of observations have been described previously in the literature (21, 26, 39, 42, 47). However, we should point out that in our FBN rat model of advanced aging, the slowing of LV relaxation was quite subtle relative to that observed in other rat models of aging (19, 45). In addition, we found no evidence of age- or training-induced alterations in [Ca\(^{2+}\)], dynamics during electrical pacing of single cardiocytes. The absence of gross age-associated alterations in [Ca\(^{2+}\)], dynamics in paced cardiocytes is at odds with published work from other laboratories (16, 44). Several studies have demonstrated [Ca\(^{2+}\)] transient prolongation with advanced aging in electrically paced papillary muscle and isolated cardiocyte preparations (28, 33). In general, it is thought that the prolongations of myocardial relaxation times that are typically observed in aged preparations (5, 39, 45) occur as a result of slowed [Ca\(^{2+}\)] clearance from the sarcoplasm caused by a diminished expression and activity of the cardiac isoform of the sarco(endo)plasmic reticular Ca\(^{2+}\) ATPase (SERCA2) (40). It is relevant to note, however, that none of the studies that demonstrated large age-associated reductions in lusitropic function and SERCA2 activity, as well as [Ca\(^{2+}\)] transient prolongation, used the FBN rat model of aging. Contrary to this previous body of work, Wahr et al. (43) concluded that SR function was maintained in advanced aging in intact FBN cardiocytes. The fact that we only observed a subtle diminution in LV lusitropic function and did not detect alterations in paced cardiocyte shortening or [Ca\(^{2+}\)], dynamics is consistent with this point of view. The FBN rat strain exhibits less systemic and cardiovascular pathology with age and maintains normal cardiac function until later in life than other rat strains (43). Therefore, it seems reasonable that age-associated alterations in lusitropic function and Ca\(^{2+}\) regulation in the absence of overt animal pathology are less prominent or absent in FBN rats. We did, however, observe action potential prolongation in the aged FBN rat model, giving rise to the interesting possibility that action potential prolongation is a true age-related phenomenon, whereas reduced SR Ca\(^{2+}\) clearance occurs as a result of pathologies secondary to the aging process.

There are several other issues regarding the results of our studies warranting comment. First, although we did observe subtle lusitropic dysfunction in our isovolumic left heart preparation, we found no evidence for slowed Ca\(^{2+}\) clearance or mechanical relaxation in unloaded single myocytes. We believe that there is a simple explanation for this apparent paradox that centers on data variability inherent in different measurement techniques. The coefficients of variation of “relaxation” data derived from isovolumic heart pressure and fura 5F-reported [Ca\(^{2+}\)] transient measurements were ~3–6% and ~30–50%, respectively. The small changes in late relaxation that were observed in the isovolumic heart studies would have been undetectable in our single-cell studies. Second, because we did observe an age-related increase (~30–40%) in forward Na\(^{+}/\)Ca\(^{2+}\) exchange-mediated Ca\(^{2+}\) clearance from myocytes studied under strict voltage control, the question arises as to why these changes were not manifest as alterations in the [Ca\(^{2+}\)] decline phases in our electrically paced myocyte and caffeine contraction experiments. With regard to the latter, inspection of Table 4 reveals that the \(k_{\text{efflux}}\) values for the AS and AT cardiocytes were ~10–20% higher than for YS cardiocytes, but owing to the high variability inherent in fura 5F data, we were not able to determine whether this apparent age-related \(k_{\text{efflux}}\) trend was reflective of pure chance or of a true increase in a Ca\(^{2+}\) clearance mechanism. As pointed out above, we believe that it was only under conditions with the strictest experimental control that age-related Na\(^{+}/\)Ca\(^{2+}\) exchange-mediated Ca\(^{2+}\) clearance differences were statistically observable.
Finally, the question arises as to why age-related increases in Na\(^+/\)Ca\(^{2+}\) exchange activity were not discernible in the shape of [Ca\(^{2+}\)] transients recorded from electrically paced cells. In electrically paced cardiocytes, the rate constant of [Ca\(^{2+}\)] decline occurs at \(\sim 6.5 \text{ s}^{-1}\) (see Table 3), whereas Ca\(^{2+}\) clearance via Na\(^+/\)Ca\(^{2+}\) exchange occurs at \(\sim 1 \text{ s}^{-1}\) (see k\(_{\text{Ca}}\) data in Table 4 and Fig. 6); the rate of Ca\(^{2+}\) clearance via Na\(^+/\)Ca\(^{2+}\) exchange is only \(\sim 15\%\) of that mediated by fast processes (i.e., the SR and intracellular buffers). In view of the very small contribution of Na\(^+/\)Ca\(^{2+}\) exchange to Ca\(^{2+}\) clearance during a single contraction-relaxation cycle, it is unlikely that a modest (\(\sim 30–40\%\)) alteration in Na\(^+/\)Ca\(^{2+}\) exchange activity would be discernable in the [Ca\(^{2+}\)] transient. This viewpoint is consistent with the observation that, in a murine model of NCX1 overexpression (49), an approximately threefold increase in Na\(^+/\)Ca\(^{2+}\) exchange activity (i.e., \(10\times\) greater than that observed in this study) only produced a 30\% acceleration in Ca\(^{2+}\) clearance during electrical pacing.

In summary, we found that in the FBN rat model of advanced age there was a very small but significant impairment in myocardial relaxation at the whole heart level that was ameliorated by a program of endurance treadmill running. This age-related impairment or its amelioration by training is not mediated by intrinsic alterations in Na\(^+/\)Ca\(^{2+}\) exchange. In single LV cardiocytes studied under highly controlled conditions, we found evidence of an increase in forward Na\(^+/\)Ca\(^{2+}\) exchange activity that was uninfuenced by exercise training. This occurred in the absence of age- or training-induced changes in NCX1 expression in any region of the heart. The increases in Na\(^+/\)Ca\(^{2+}\) exchange activity that we observed were associated with, and provide a possible explanation for, late phase action potential prolongation in advanced age.

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DISCLOSURES

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