Overexpression of type 1 angiotensin II receptors impairs excitation-contraction coupling in the mouse heart

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Overexpression of type 1 angiotensin II receptors impairs excitation-contraction coupling in the mouse heart. Am J Physiol Heart Circ Physiol 301: H2018–H2027, 2011. First published August 26, 2011; doi:10.1152/ajpheart.01092.2010.—Transgenic mice that overexpress human type 1 angiotensin II receptor (AT1R) in the heart develop cardiac hypertrophy. Previously, we have shown that in 6-mo AT1R mice, which exhibit significant cardiac remodeling, fractional shortening is decreased. However, it is not clear whether altered contractility is attributable to AT1R overexpression or is secondary to cardiac hypertrophy/remodeling. Thus the present study characterized the effects of AT1R overexpression on ventricular L-type Ca2+ currents (ICaL), cell shortening, and Ca2+ handling in 50-day and 6-mo-old males AT1R mice. Echocardiography showed there was no evidence of cardiac hypertrophy in 50-day AT1R mice but that fractional shortening was decreased. Cellular experiments showed that cell shortening, ICaL, and Ca1.2 mRNA expression were significantly reduced in 50-day and 6-mo-old AT1R mice compared with controls. In addition, Ca2+ transients and caffeine-induced Ca2+ transients were reduced whereas the time at 90% Ca2+ transient decay was prolonged in both age groups of AT1R mice. Western blot analysis revealed that sarcoplasmic reticulum Ca2+-ATPase and Na+/Ca2+ exchanger protein expression was significantly decreased in 50-day and 6-mo AT1R mice. Overall, the data show that cardiac contractility and the mechanisms that underlie excitation-contraction coupling are altered in AT1R mice. Furthermore, since the alterations in contractility occur before the development of cardiac hypertrophy, it is likely that these changes are attributable to the increased activity of the renin-angiotensin system brought about by AT1R overexpression. Thus it is possible that AT1R blockade may help maintain cardiac contractility in individuals with heart disease.

THE RENIN-ANGIOTENSIN SYSTEM (RAS) regulates important cardiovascular processes, such as blood pressure and volume as well as cell proliferation and growth (31). Increased RAS activity is associated with a number of cardiac pathologies. These include cardiac hypertrophy, which is an increase in cardiac myocyte size, and ventricular remodeling, which is the alteration of the structure of the heart (e.g., dimension, mass, and/or shape; Refs. 11, 17). RAS also contributes to the development and progression of heart failure (2, 35). The primary RAS effector is angiotensin II (ANG II; Ref. 31), which primarily acts on the type 1 ANG II receptor (AT1R; Ref. 12). ANG II can alter the phenotype and morphology of several cell types, such as endothelial cells, fibroblasts, and cardiac myocytes (31), altering cardiac structure and function. These changes can lead to the development and progression of heart failure. As a result, several transgenic mouse models that overexpress different components of the RAS have been generated to help elucidate the role of RAS in the development and progression of cardiac failure (15, 30, 34).

AT1R transgenic mice overexpress the human AT1R specifically in the heart (34). These mice develop cardiac hypertrophy and undergo cardiac remodeling, both of which occur independently of hemodynamic changes (34, 37). Over time physiological and biochemical changes occur within the hypertrophied heart and lead to cardiac dysfunction, such as alterations in cardiac contractility (26, 29), and ultimately heart failure. Interestingly, several studies (23, 38, 41) have shown that AT1R blockade can counteract cardiac hypertrophy, remodeling, and improve cardiac contractility. This suggests that the actions of ANG II on AT1R may modulate cardiac contractility.

Cardiac contractile function is initiated by a transient increase in intracellular Ca2+ ([Ca2+]i) (5). Excitation of the sarcolemma results in extracellular Ca2+ entering the cell via L-type Ca2+ channels, resulting in an L-type Ca2+ current (ICaL), which triggers the release of Ca2+ from the sarcoplasmic reticulum (SR). This results in a transient increase in [Ca2+]i, which activates the myofilaments and enables contraction to occur. This process is known as excitation-contraction coupling (ECC; Ref. 5). Relaxation occurs as Ca2+ is removed from the cytosol and [Ca2+]i decreases. Removal of Ca2+ from the cytosol is facilitated by the SR Ca2+-ATPase (SERCA2a) and the Na+/Ca2+ exchanger (NCX; Ref. 6). Since Ca2+ is vital for contraction, small alterations in Ca2+ handling can result in contractile dysfunction.

It is recognized that changes in the RAS can alter cardiac contractility (16, 18, 22). In fact, we (37) have previously shown that cardiac contractility is reduced in 6-mo AT1R mice (37). These mice also exhibit an increase in the left ventricular (LV) weight-to-body weight ratio and ventricular myocyte capacitance, which is indicative of myocyte hypertrophy. Thus it is not clear whether AT1R overexpression directly alters cardiac contractility or whether contractile dysfunction is secondary to AT1R overexpression-mediated hypertrophy. Our previous work (37) shows that repolarization is prolonged in young AT1R mice (50 day). These mice do not exhibit cardiac hypertrophy, which suggests that cardiac-specific AT1R overexpression, rather than cardiac hypertrophy, may contribute to alteration in cardiac function. Thus the objective of this study was to determine if cardiac contractility and the mechanisms that underlie ECC were altered in young (no cardiac hypertro-

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phypathy) and older (cardiac hypertrophy) AT1R mice as well as to determine whether alterations in contraction/ECC were attributable to AT1R overexpression or were secondary to cardiac hypertrophy and remodeling.

**METHODS**

*Animal model.* This study utilized heterozygous male C57BL/6 AT1R transgenic mice (34) aged 50 ± 5 days old (50 day) and 6–8 mo old (6 mo) as well as sex and age-matched wild-type littermate controls (CTL). All experiments were performed in accordance to the guidelines of the Canadian Council on Animal Care and 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). Experiments were also approved by the Montreal Heart Institute Animal Care Committee.

*Echocardiography.* Echocardiography was performed as previously described (3). In brief, mice were anesthetized with a 1:1 mixture (10 ml/g ip) of fentanyl (5 mg/ml) and droperidol (250 mg/g) and placed on a heated platform to maintain body temperature. Echocardiography was performed with a Philips Sonos 5500 ultrasound machine and a 15-MHz linear-array transducer (Philips Electronics, Markham, ON, Canada). Two-dimensional M-mode echocardiography was performed at the level of the papillary muscles to determine the left ventricular end-diastolic and end-systolic internal dimension. Fractional shortening was calculated as described in Yang et al. (44).

*Cell isolation.* Ventricular myocytes were isolated as previously described (19, 43). In brief, mice were heparinized (1 U/kg ip) 20 min before death to prevent blood coagulation. Mice were then anesthetized with isoflurane and killed by cervical dislocation. The chest was opened, and the heart was rapidly excised and hung on a modified Lagendorff apparatus. The heart was perfused retrogradely through the aorta (2 ml/min) with the following solutions: 1) 5 min with HEPES-buffered Tyrode solution containing (in mM): 130 NaCl, 5.4 KCl, 1 CaCl2, 1 MgCl2, 0.33 Na2HPO4, 10 HEPES, and 5.5 glucose (pH adjusted to 7.4 with NaOH); 2) 10 min with a Tyrode solution with zero Ca2+; 3) 20 min with a Tyrode solution containing 0.03 mM Ca2+, 20 mM taurine, 0.1% BSA (Fraction V; Sigma Chemicals, St Louis, MO), and 73.7 U/ml type II collagenase (Worthington, Freehold, NJ); and 4) 7.5 min with a Kraft-Brühe (KB) solution containing (in mM) 100 K-glutamate, 10 K-asparate, 25 KCl, 10 KH2PO4, 2 MgSO4, 0.33 Na2HPO4, 5.5 glucose (pH 7.4 with KOH). All solutions were maintained at 37 ± 1°C. Upon completion of perfusion, the right ventricular free wall was removed and placed in KB. Next, the tissue was minced and titrated for 10 min to isolate individual ventricular myocytes. Myocytes were stored in KB solution at 4°C until needed.

*Cell shortening.* Cells were perfused with a Tyrode solution and field stimulated at a rate of 2 Hz through a pair of bipolar platinum electrodes. Electrical pulses were generated with a Grass SD9 stimulator. Unloaded cell shortening was measured with a video edge detector (Crescent Electronics, Sandy, UT) operating at 120 Hz. Cells were visualized with a CCD video camera (MCS100 MyoCam; IonOptix, Milton, MA) and displayed on a video monitor. Contraction traces were recorded with Axoscope 8.2 (Molecular Devices, Foster City, CA). Patch pipettes (2–4 MΩ) were made with borosilicate glass (WPI, Sarasota, FL) and filled with the following solution (in mM): 80 aspartic acid, 40 CsCl, 70 CsOH, 10 EGTA, 10 HEPES, 2 MgCl2, and 4 Na2ATP (pH 7.2 CsOH). Series resistance compensation was set at 70–80%. Data were corrected for a 10-mV liquid junction potential. Voltage-clamp recordings were low-pass filtered (1 kHz) and digitized (4 kHz) and stored on a computer. pCLAMP 8.2 was used to generate voltage protocols and record data. The voltage protocol used to initiate I_Ca,L is illustrated in RESULTS (see Fig. 2). Patch-clamp experiments were conducted at room temperature (20–22°C).

*Real time RT-PCR.* Real-time RT-PCR [quantitative (q)PCR] for the α-subunit of the L-type Ca2+ channel (Ca1.2) was conducted using previously published protocols (21, 24). In brief, total RNA was isolated from the ventricles with a RNeasy fibrous tissue kit (Qiagen) and treated with DNase I to prevent contamination by genomic DNA. cDNA was then synthesized with the cloned AMV reverse transcriptase (Invitrogen) and primers specific for Ca1.2 (24). The qPCR was performed with Platinum SYBR Green qPCR Supermix (Invitrogen) using a real-time PCR system (MX3005P QPCR system; Stratagene). mRNA expression was quantified relative to the level of murine cyclophilin mRNA.

*Ca2+ transients and caffeine-induced Ca2+ transients.* The protocols utilized for the Ca2+ transient and SR Ca2+ content experiments were similar to those used by Grady and Howlett (20). In brief, ventricular myocytes were incubated in the dark (20–22°C) with 10 μM fluo-4 (Molecular Probes). Myocytes were then placed in an experimental chamber on the stage of a Zeiss LSM 510 laser scanning microscope, perfused with a Tyrode solution. To assess Ca2+ transients, myocytes were continuously field stimulated at a rate of 2 Hz with a pair of bipolar platinum electrodes (Grass SD9 stimulator). To assess SR Ca2+ content, ventricular myocytes were field stimulated at a frequency of 2 Hz for 10 consecutive contractions (conditioning pulses) to ensure that each cell had a similar activation history before the application of caffeine. Upon completion of the conditioning pulses, 10 μM caffeine were rapidly applied to the myocyte for 10 s via a rapid solution switcher. In both sets of experiments, changes in free Ca2+ were measured in line scan mode with excitation at 488 nm and emission measured at 505–530 nm. Myocytes were scanned repeatedly along the length of the cell at 1.5-ms intervals for 7 s. Sequential scans were stacked to create a two-dimensional image. Image J (NIH) was used to visualize the Ca2+ transients, and the data were analyzed with pCLAMP 8.2. Experiments to examine Ca2+ transients and SR Ca2+ content were conducted at physiological temperature (37°C).

*Western blot analysis.* The protocols used to isolate the enriched total membrane protein fraction as well as the Western blot analysis were identical to those previously reported (8, 27, 43). In brief, total membrane proteins were isolated from the ventricles of control and AT1R mice (1 heart/sample). Proteins (100 μg/lane) were separated by SDS-PAGE and electrophoretically transferred onto a PVDF membrane. Ponceau S staining was used to confirm the uniformity of protein transfer. Membranes were then assayed to determine levels of protein expression. Rabbit polyclonal primary antibodies were directed against SERCA2a (1:1,000; Santa Cruz) and NCX (1:500; Affinity Bioreagents). Immunoreactive bands were quantified by densitometry (Multi-Analyist program; Bio-Rad, CA). Protein expression was normalized to GAPDH.

*Data analysis.* Results are expressed as means ± SE. An unpaired Student *t*-test was used to compare means. A *P* value of <0.05 was considered statistically significant.

**RESULTS**

Cardiac structure and function are altered in AT1R mice. Previously, we (37) have shown that fractional shortening is significantly reduced in the presence of ventricular remodeling in 6-mo AT1R mice. However, it was not clear whether altered...
contractility was attributable to AT1R overexpression or was secondary to cardiac hypertrophy and remodeling. Initial studies (37) with 50-day AT1R mice revealed that there was no evidence of cellular hypertrophy. In addition, visual inspection suggests that hypertrophy was not present in the 50-day AT1R heart (37). To substantiate these observations, we conducted echocardiography to confirm cardiac structure was unaltered in 50-day AT1R mice. The results presented in Table 1 show that the LV weight-to-body weight ratio, LV internal diameter during diastole and systole, interventricular septum thickness, and LV posterior wall thickness did not differ significantly between 50-day CTL and AT1R mice. Thus there were no overt signs of ventricular hypertrophy in 50-day AT1R mice. Next, we examined fractional shortening in 50-day AT1R mice to determine if AT1R overexpression affected contractility in the absence of cardiac hypertrophy. The results indicate that fractional shortening was significantly reduced in 50-day AT1R mice compared with age-matched controls (Table 1). Together these findings suggest that cardiac AT1R overexpression directly contributes to the reduction in cardiac contractility observed in 50-day AT1R mice.

Table 1. Effect of AT1R overexpression on left ventricular structure and function in 50-day-old mice

<table>
<thead>
<tr>
<th>Parameters</th>
<th>CTL</th>
<th>AT1R</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell capacitance</td>
<td>150 ± 7</td>
<td>156 ± 10</td>
<td>NS</td>
</tr>
<tr>
<td>Body wt, g</td>
<td>20.3 ± 0.9</td>
<td>20.6 ± 0.6</td>
<td>NS</td>
</tr>
<tr>
<td>LV mass, mg</td>
<td>98.1 ± 10.4</td>
<td>102.9 ± 0.1</td>
<td>NS</td>
</tr>
<tr>
<td>LV mass/body wt, mg/g</td>
<td>4.8 ± 0.4</td>
<td>5.0 ± 0.2</td>
<td>NS</td>
</tr>
<tr>
<td>IVSd, mm</td>
<td>9.3 ± 0.5</td>
<td>7.5 ± 0.2</td>
<td>NS</td>
</tr>
<tr>
<td>LVPWd, mm</td>
<td>9.7 ± 1.1</td>
<td>8.2 ± 1.5</td>
<td>NS</td>
</tr>
<tr>
<td>LVIDd, mm</td>
<td>30.4 ± 0.7</td>
<td>37.6 ± 4.5</td>
<td>NS</td>
</tr>
<tr>
<td>LVIDs, mm</td>
<td>14.7 ± 1.0</td>
<td>24.9 ± 2.1</td>
<td>NS</td>
</tr>
<tr>
<td>FS, %</td>
<td>51.6 ± 1.9</td>
<td>33.6 ± 2.3</td>
<td>0.007</td>
</tr>
</tbody>
</table>

Values are means ± SE. Echocardiography was used to measure left ventricular (LV) internal diameter during diastole and systole (LVIDd and LVIDs, respectively), interventricular septum thickness (IVSd), and posterior wall thickness (LVPWd) and to calculate the LV mass/body wt index. Echocardiography also was used to assess LV systolic function (FS). CTL, control; AT1R, type 1 angiotensin II receptor mice.

To further investigate the effects of AT1R overexpression on cardiac contractility, unloaded cell shortening was measured in ventricular myocytes from 50-day and 6-mo CTL and AT1R mice. Representative traces show that contraction amplitude was decreased in myocytes from 50-day and 6-mo AT1R mice compared with age-matched controls (Fig. 1, A and C). Contraction amplitudes were normalized to cell length and were expressed as percent cell shortening. The mean data show that percent cell shortening was significantly reduced in myocytes from 50-day and 6-mo AT1R mice compared with controls (Fig. 1, B and D). Overall, these results suggest that AT1R overexpression alters cardiac contractility at the cellular level. Of note, cell length was similar in CTL and AT1R myocytes in both age groups (50 day: CTL 129.4 ± 0.4 μm, AT1R 140.0 ± 6.2 μm, P = NS; 6 mo: CTL 124.0 ± 2.5 μm, AT1R 132.1 ± 3.7 μm, P = NS). Interestingly, cell capacitance was only increased in myocytes from 6-mo AT1R mice (see below). Thus the increase in cell capacitance is likely attributable to an increase in cell width rather than length. 

ICaL was decreased in ventricular myocytes from AT1R mice. The focus of the next series of experiments was to determine the effects of AT1R overexpression, in the absence (50 day)
and presence (6 mo) of cardiac hypertrophy, on the mechanisms that underlie ECC. Since \( I_{\text{CaL}} \) is the primary trigger for calcium-induced calcium release, we first characterized \( I_{\text{CaL}} \) in ventricular myocytes from 50-day and 6-mo AT\(_1\)R mice. Representative current traces show that \( I_{\text{CaL}} \) was decreased in ventricular myocytes isolated from 50-day and 6-mo AT\(_1\)R mice (Fig. 2, A and C). \( I_{\text{CaL}} \) was normalized to cell capacitance and expressed as current densities (pA/pF). Cell capacitance was similar for 50-day CTL and AT\(_1\)R myocytes (Table 1), whereas cell capacitance was significantly greater in myocytes from 6-mo AT\(_1\)R mice compared with age-matched controls (50 day: CTL 2.0 ± 0.1, \( n = 13 \); AT\(_1\)R 1.7 ± 0.04, \( n = 13 \), \( P < 0.05 \); 6 mo: CTL 2.2 ± 0.1, \( n = 20 \); AT\(_1\)R 1.9 ± 0.1, \( n = 14 \), \( P < 0.05 \)). The mean current-voltage relationships show that \( I_{\text{CaL}} \) (Fig. 2B, D) was significantly reduced in 50-day and 6-mo AT\(_1\)R ventricular myocytes compared with their respective controls (at 0 mV: 50 day, CTL −7.4 ± 0.38 pA/pF, AT\(_1\)R −4.2 ± 0.23 pA/pF; 6 mo, CTL −5.9 ± 0.23 pA/pF, AT\(_1\)R −3.4 ± 0.20 pA/pF). This suggests that cardiac-specific overexpression of AT\(_1\)R has a marked effect on ventricular \( I_{\text{CaL}} \), independent of cardiac hypertrophy and remodeling.

Subsequently, we examined ventricular Cav1.2 mRNA expression, which underlies \( I_{\text{CaL}} \), to determine if the reduction in \( I_{\text{CaL}} \) was attributable to alterations in Ca\(^{2+}\) channel expression. Figure 3 shows that Cav1.2 mRNA expression was significantly decreased in the ventricles of 50-day (Fig. 3A) and 6-mo (Fig. 3B) AT\(_1\)R mice (\( P < 0.05 \)). Thus the reduction in \( I_{\text{CaL}} \) density is likely attributable to a decrease in Cav1.2 mRNA expression.

Peak Ca\(^{2+}\) transient amplitude was decreased in ventricular myocytes from AT\(_1\)R mice. Since the size of the Ca\(^{2+}\) transient is graded by the magnitude of \( I_{\text{CaL}} \) (7), we next examined the effect of AT\(_1\)R overexpression on SR Ca\(^{2+}\) release. Representative series of Cav\(^{2+}\) transients from CTL and AT\(_1\)R myocytes are shown in Fig. 4, A and D. The mean data revealed that the Ca\(^{2+}\) transient amplitude was significantly decreased in myocytes from 50-day (Fig. 4B) and 6-mo (Fig. 4E) AT\(_1\)R mice compared with age-matched controls (50 day: CTL 2.0 ± 0.1, \( n = 13 \); AT\(_1\)R 1.7 ± 0.04, \( n = 13 \), \( P < 0.05 \); 6 mo: CTL 2.2 ± 0.1, \( n = 20 \); AT\(_1\)R 1.9 ± 0.1, \( n = 14 \), \( P < 0.05 \)). In addition, the time to 90% transient decay was significantly increased in myocytes from 50-day (Fig. 4C) and 6-mo (Fig. 4F) AT\(_1\)R mice (50 day: CTL 0.25 ± 0.01 s, \( n = 13 \); AT\(_1\)R 0.27 ± 0.01 s, \( n = 13 \), \( P < 0.05 \); 6 mo: CTL 0.24 ± 0.01 s, \( n = 20 \); AT\(_1\)R 0.26 ± 0.01 s, \( n = 14 \), \( P < 0.05 \)). Overall, the data suggest that AT\(_1\)R overexpression alters SR Ca\(^{2+}\) release in 50-day and 6-mo AT\(_1\)R mice as well as contributes to alterations in SR Ca\(^{2+}\) reuptake.

SR Ca\(^{2+}\) content was decreased in ventricular myocytes from AT\(_1\)R mice. The reduction in the amplitude of Ca\(^{2+}\) transients in ventricular myocytes from 50-day and 6-mo AT\(_1\)R may be attributable to the reduction in \( I_{\text{CaL}} \). However, the change in transient amplitude also could be attributable to a reduction in SR Ca\(^{2+}\) content. Therefore, the next series of

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**Fig. 2.** Ventricular L-type Ca\(^{2+}\) current (\( I_{\text{CaL}} \)) in 50-day and 6-mo AT\(_1\)R mice. Representative ventricular \( I_{\text{CaL}} \) traces from 50-day (A) and 6-mo (C) CTL and AT\(_1\)R mice. Inset: cells were held at −80 mV and 250-ms test steps were made in 10-mV increments from −50 mV to +60 mV at a rate of 0.1 Hz to activate membrane currents. All test steps were preceded by a 50-ms prepulse to −45 mV to inactivate Na\(^{+}\) current. Mean current-voltage curves for \( I_{\text{CaL}} \) from 50-day (B) and 6-mo (D) CTL and AT\(_1\)R ventricular myocytes (*\( P < 0.05 \); CTL: 50 day \( n = 20 \), 6 mo \( n = 17 \); AT\(_1\)R: 50 day \( n = 18 \), 6 mo \( n = 15 \)).
experiments quantified and compared SR Ca\textsuperscript{2+} content in ventricular myocytes from 50-day and 6-mo CTL and AT\textsubscript{1}R mice. Representative examples of caffeine-induced Ca\textsuperscript{2+} transients from myocytes isolated from 50-day and 6-mo CTL and AT\textsubscript{1}R mice are shown in Fig. 5, A and D, respectively. The mean data show that caffeine-induced Ca\textsuperscript{2+} transients were significantly smaller in AT\textsubscript{1}R myocytes from both age groups compared with age-matched controls (50 day: CTL 4.2 ± 0.3, n = 13; AT\textsubscript{1}R 2.9 ± 0.2, n = 12, P < 0.05; 6 mo: CTL 3.8 ± 0.2, n = 14; AT\textsubscript{1}R 3.0 ± 0.1, n = 14, P < 0.05). Similar to the Ca\textsuperscript{2+} transients, the time to 90% decay of the caffeine-induced Ca\textsuperscript{2+} transients was prolonged in 6-mo AT\textsubscript{1}R myocytes compared with age-matched controls (Fig. 5F; CTL 10.7 ± 1.6 s, n = 14; AT\textsubscript{1}R 17.7 ± 2.1 s, n = 14; P < 0.05). However, the prolongation of time to 90% decay was not statistically significant in 50-day AT\textsubscript{1}R myocytes (Fig. 5C; CTL 13.2 ± 1.7 s, n = 13; AT\textsubscript{1}R 16.6 ± 1.3 s, n = 12; P < 0.05). Overall, the results suggest that SR Ca\textsuperscript{2+} content is reduced in ventricular myocytes from AT\textsubscript{1}R myocytes independent of age. This decrease in SR Ca\textsuperscript{2+} content likely contributes to the observed reduction in SR Ca\textsuperscript{2+} release observed in AT\textsubscript{1}R myocytes.

Alterations in Ca\textsuperscript{2+} handling proteins in AT\textsubscript{1}R mice. Changes in the removal of calcium from the cytosol also could affect contractility. Thus Western blot analysis was performed to determine if SERCA2a and/or NCX expression was altered in AT\textsubscript{1}R mice. Figure 6 shows that SERCA2a expression was significantly decreased in the ventricles of both 50-day (Fig. 6A) and 6-mo (Fig. 6B) AT\textsubscript{1}R mice compared with age-matched controls. In addition, NCX expression was significantly reduced in 50-day and 6-mo AT\textsubscript{1}R mice (Fig. 7, A and B).

**DISCUSSION**

ANG II has been shown to contribute to the progression of heart failure (2, 35). More specifically, ANG II has a negative inotropic effect (33), which may contribute to contractile dysfunction that is associated with heart failure. Previously, we (37) have shown that 6-mo male AT\textsubscript{1}R mice develop both cardiac hypertrophy and ventricular contractile dysfunction. Similarly, the present study showed that myocyte contractile function was also decreased in 6-mo AT\textsubscript{1}R mice. However, based on the finding obtained in 6-mo mice it was not clear whether changes in contractility were the result of AT\textsubscript{1}R overexpression or secondary to cardiac hypertrophy. The present study showed that in 50-day AT\textsubscript{1}R mice, which do not exhibit cardiac hypertrophy, both fractional shortening and myocyte contractility were decreased compared with age-matched controls. In addition, the present study shows that AT\textsubscript{1}R overexpression altered the mechanisms that underlie cardiac contractation. Specifically, results showed that I\textsubscript{CaL}, Ca\textsuperscript{2+} transients, and SR Ca\textsuperscript{2+} content were significantly reduced and the time to 90% Ca\textsuperscript{2+} transient decay was prolonged in ventricular myocytes from 50-day and 6-mo AT\textsubscript{1}R mice. In addition, SERCA2a and NCX expression were significantly decreased in ventricular tissue from both age groups of AT\textsubscript{1}R mice. The reduction in I\textsubscript{CaL} and SR Ca\textsuperscript{2+} content is likely responsible for decrease in SR Ca\textsuperscript{2+} release and the subsequent reduction in cardiac contractility in 50-day and 6-mo AT\textsubscript{1}R mice. Thus it appears that AT\textsubscript{1}R overexpression is primarily responsible for the altered contractile phenotype observed in AT\textsubscript{1}R rather than the changes being secondary to cardiac hypertrophy and remodeling.

**ECC is altered in AT\textsubscript{1}R mice.** Several studies (16, 22) have shown that alterations in the RAS can alter ECC. These studies have focused specifically on the effects on ANG II. For example, several studies have shown that the treatment of isolated cardiomyocytes with ANG II has a negative inotropic effect (32, 33). Similarly, Domenighetti et al. (16) showed that ANG II overexpression alters cardiac contractility. However, in these studies alterations in ECC occurred in conjunction with cardiac hypertrophy. Thus it is not clear whether decreased cardiac contractility is attributable to ANG II overexpression or secondary to cardiac hypertrophy. The present study is the first study, to the authors’ knowledge, to show cardiac-specific overexpression of AT\textsubscript{1}R decreases cardiac and ventricular myocyte contractility in the absence of cardiac hypertrophy. This demonstrates that increases in AT\textsubscript{1}R independent of changes in ANG II levels can alter cardiac contractility.
To determine the mechanism by which AT1R overexpression alters cardiac contractility, we first examined the L-type Ca\textsuperscript{2+} current, which initiates the process of Ca\textsuperscript{2+} induced Ca\textsuperscript{2+} release and leads to the contraction of the myocyte. The results from the present study show that I\textsubscript{CaL} was significantly reduced in ventricular myocytes from both 50-day and 6-mo AT1R mice. This reduction is likely the result of the decrease in Cav1.2 mRNA expression in AT1R mice and the subsequent reduction in L-type channel protein expression. Of note, some studies have shown that ANG II has the opposite effect on I\textsubscript{CaL} (13, 22). In particular, Gusev et al. (22) reported small increase in I\textsubscript{CaL} in 5-mo-old transgenic mice with cardiac-specific overexpression of ANG II. The different effects of AT1R and ANG II on I\textsubscript{CaL} may be attributable to differences in ANG II concentrations in the experimental models. In the ANG II overexpression model, ANG II is very abundant and may activate signaling pathways unrelated to the AT1R. These signaling mechanisms could be responsible for the increase in I\textsubscript{CaL} reported by Gusev et al. (22). In the AT1R overexpression model, the plasma concentration ANG II is similar in CTL and AT1R mice (unpublished data). Thus it is less likely that ANG II will activate alternate signaling pathways. Overall, results presented here demonstrate that AT1R overexpression alters L-type Ca\textsuperscript{2+} channel expression, which results in a decrease in I\textsubscript{CaL}.

As previously mentioned, I\textsubscript{CaL} is a key determinant of the magnitude of SR Ca\textsuperscript{2+} release (10, 28). Thus it would be expected that a reduction in I\textsubscript{CaL} would alter the amplitude of the Ca\textsuperscript{2+} transient. This is confirmed by the present data that clearly show that the Ca\textsuperscript{2+} transient amplitude was markedly reduced in ventricular myocytes from 50-day and 6-mo AT1R mice. The present study also showed that the decay phase of the Ca\textsuperscript{2+} transient was significantly prolonged in myocytes from AT1R mice compared with age-matched controls. This suggests that Ca\textsuperscript{2+} reuptake and/or removal of Ca\textsuperscript{2+} from the cell may be altered in cardiac myocytes from AT1R mice.

In the mouse heart, reuptake and removal of Ca\textsuperscript{2+} from the cytosol are primarily attributable to SERCA2a. Previous studies (16, 22) have shown that ANG II overexpression decreases SERCA2a expression. Similarly, the current study showed that SERCA2a expression in the ventricles was significantly reduced in 50-day and 6-mo AT1R mice. The decrease in SERCA2a expression in the ventricles likely results in a reduction in Ca\textsuperscript{2+} reuptake and the subsequent delay in Ca\textsuperscript{2+} transient decay. SERCA2a also plays an important role in the regulation of SR Ca\textsuperscript{2+} content (1). For example, Gusev et al. (22) reported that SERCA2a expression, SR Ca\textsuperscript{2+} content, and SR Ca\textsuperscript{2+} release are reduced in mice that overexpress ANG II. Furthermore, knockout of the SERCA gene has been shown to reduce SR Ca\textsuperscript{2+} content and SR Ca\textsuperscript{2+} release (1). Therefore, the
present study showed that SR Ca\(^{2+}\) content was decreased in both age groups of AT\(_1\)R mice. Thus the reduction in SERCA2a expression in AT\(_1\)R hearts is likely responsible for the reduction in SR Ca\(^{2+}\) content. Since SR Ca\(^{2+}\) content is a key determinant of SR Ca\(^{2+}\) release (4), decreased Ca\(^{2+}\) content could contribute to the reduction in SR Ca\(^{2+}\) release in AT\(_1\)R mice. The decrease in SR Ca\(^{2+}\) release could also be partially responsible for the reduction in cardiac contractility observed in AT\(_1\)R mice.

NCX also contributes to the removal of Ca\(^{2+}\) from the cytosol during diastole (42). In the present study, the decay phase of caffeine-induced Ca\(^{2+}\) transients was prolonged in myocytes from AT\(_1\)R mice, which suggests that Ca\(^{2+}\) extrusion via NCX is reduced. This reduction in NCX function is likely attributable to decreased NCX expression in ventricular myocytes from AT\(_1\)R mice. In addition, ventricular I\(_{\text{CaL}}\) was decreased in AT\(_1\)R mice. Similarly, ventricular I\(_{\text{CaL}}\) is reduced in NCX knockout mice (36). It is believed that the reduction in I\(_{\text{CaL}}\) is in response to decreased NCX expression. This adaptation occurs to prevent Ca\(^{2+}\) overload in the myocyte (36). Thus, in AT\(_1\)R mice, the reduction in NCX likely contributes to the delay in Ca\(^{2+}\) removal from the cytosol as well as the reduction in I\(_{\text{CaL}}\) and the subsequent alterations observed in ECC.

**ANG II is responsible for alterations in ventricular Ca\(^{2+}\) handling.** Previous studies (16, 22) reported that mice with cardiac-specific overexpression of ANG II exhibit cardiac hypertrophy, decreased contractility, and alterations in Ca\(^{2+}\) handling. Similarly, we have previously reported that 6-mo AT\(_1\)R mice develop ventricular hypertrophy, cardiac arrhythmia, and repolarization defects (37). However, we reported that the increased incidence of arrhythmia and delayed repolarization also occurred in 50-day AT\(_1\)R mice that do not present signs of hypertrophy. Thus our previous work strongly suggested that alterations in cardiac function could be directly attributable to increased activity of the RAS. In support of that notion, results reported here also show that changes in ECC and contractility are similar in 50-day and 6-mo AT\(_1\)R mice. These findings confirm that the alterations in ECC and contractility are not secondary to cardiac hypertrophy but are the result of AT\(_1\)R overexpression.

Studies (14, 25, 39) have shown that alterations in the action potential waveform can also affect ECC and contractility. Interestingly, we have previously shown that AT\(_1\)R overexpression prolongs ventricular repolarization in both 50-day and 6-mo AT\(_1\)R mice (37). In rodents, prolonged repolarization times are associated with a decrease in peak I\(_{\text{CaL}}\) as well as an increase in Ca\(^{2+}\) transient amplitude (25, 39, 40). Action potential duration has also been shown to affect NCX expression, whereby NCX expression is decreased in cardiac cells that have slower rates of repolarization compared with cardiac cells that have faster rates of repolarization (14). Similar to previous studies, we observed that peak I\(_{\text{CaL}}\) and NCX expres-
sion were decreased in both age groups of AT1R mice. However, our results showed that Ca^{2+} transient amplitudes were decreased in AT1R mice. This finding is in contrast to what would be expected based on an increase in action potential duration. We attribute this reduction in SR Ca^{2+} release to the decreased SR Ca^{2+} content in the AT1R myocytes. The decrease in SR Ca^{2+} content is likely the result of a reduction in SERCA2a expression and the fact that AT1R overexpression did not prolong the time course for \( I_{CaL} \). Hence, although the effects of AT1R overexpression on action potential duration may play a role in the modulation of ECC in AT1R mice, it appears that the deleterious effects of AT1R overexpression on Ca^{2+} handling are great enough that they cannot be compensated by the prolongation of the APD.

**Conclusion.** The present work shows that dramatic alterations in ECC and in major Ca^{2+} regulatory proteins result in a decrease in cell shortening in AT1R mice. These changes at the cellular level are likely responsible for the observed reduction in fractional shortening in both 50-day and 6-mo AT1R mice. Furthermore, in 50-day mice changes in contraction, ECC and Ca^{2+} handling proteins occurred before the development of ventricular hypertrophy. This clearly suggests that AT1R overexpression can alter EC coupling in the heart, independently of cardiac hypertrophy and remodeling. Overall, this shows that increased activity of the RAS, brought about by AT1R overexpression, alters cardiac contractility, which could contribute to the progression of heart failure.

![Fig. 6. SR Ca^{2+}-ATPase (SERCA2a) expression in ventricles from CTL and AT1R mice. Western blots show SERCA2a expression in 50-day (A) and 6-mo (B) CTL and AT1R mice. NS, nonspecific. SERCA2a was normalized to GAPDH (*\( P < 0.05 \); CTL: 50 day \( n = 3 \), 6 mo \( n = 3 \); AT1R: 50 day \( n = 3 \), 6 mo \( n = 3 \)).](image)

![Fig. 7. Na^{+}/Ca^{2+} exchanger (NCX) expression in the ventricles of AT1R and CTL mice. Western blots show NCX expression in 50-day (A) and 6-mo (B) CTL and AT1R mice. NCX was normalized to GAPDH (*\( P < 0.05 \); CTL: 50 day \( n = 3 \), 6 mo \( n = 3 \); AT1R: 50 day \( n = 3 \), 6 mo \( n = 3 \)).](image)
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DISCLOSURES
No conflicts of interest, financial or otherwise, are declared by the author(s).

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