Enhanced basal contractility but reduced excitation-contraction coupling efficiency and β-adrenergic reserve of hearts with increased Cav1.2 activity

Mingxin Tang,1* Xiaoying Zhang,1* Yingxin Li,1 Yinzheng Guan,1 Xiaojie Ai,1,4 Christopher Szeto,1 Hiroyuki Nakayama,2 Hongyu Zhang,1 Shuping Ge,3 Jeffery D. Molkentin,2 Steven R. Houser,1 and Xiongwen Chen1

1Cardiovascular Research Center and Department of Physiology, Temple University School of Medicine, Philadelphia, Pennsylvania; 2Cincinnati Children’s Hospital Medical Center and Department of Pediatrics, University of Cincinnati, Cincinnati, Ohio; 3St. Christopher’s Hospital for Children/Drexel University College of Medicine, Philadelphia, Pennsylvania; and 4School of Agriculture and Biology, Shanghai Jiao Tong University, Shanghai, People’s Republic of China

Submitted 15 March 2010; accepted in final form 10 June 2010

Tang M, Zhang X, Li Y, Guan Y, Ai X, Szeto C, Nakayama H, Zhang H, Ge S, Molkentin JD, Houser SR, Chen X. Enhanced basal contractility but reduced excitation-contraction coupling efficiency and β-adrenergic reserve of hearts with increased Cav1.2 activity. Am J Physiol Heart Circ Physiol 299: H519–H528, 2010. First published June 11, 2010; doi:10.1152/ajpheart.00265.2010.—Cardiac remodeling during heart failure development induces a significant increase in the activity of the L-type Ca2+ channel (Cav1.2). However, the effects of enhanced Cav1.2 activity on myocyte excitation-contraction coupling (E-C) coupling, cardiac contractility, and its regulation by the β-adrenergic system are not clear. To recapitulate the increased Cav1.2 activity, a double transgenic (DTG) mouse model overexpressing the Cavβ2a subunit in a cardiac-specific and inducible manner was established. We studied cardiac (in vivo) and myocyte (in vitro) contractility at baseline and upon β-adrenergic stimulation. E-C coupling efficiency was evaluated in isolated myocytes as well. The following results were found: 1) in DTG myocytes, L-type Ca2+ current (ICaL) density, myocyte fractional shortening (FS), peak Ca2+ transients, and sarcoplasmic reticulum (SR) Ca2+ content (caffeine-induced Ca2+ transient peak) were significantly increased (by 100.8%, 48.8%, 49.8%, and 46.8%, respectively); and 2) cardiac contractility evaluated with echocardiography [ejection fraction (EF) and (FS)] and invasive intra-left ventricular pressure (maximum dP/dt) measurements were significantly greater in DTG mice than in control mice. However, 1) the cardiac contractility (EF, FS, dP/dt, and −dP/dt)–enhancing effect of the β-adrenergic agonist isoproterenol (2 μg/g body wt ip) was significantly reduced in DTG mice, which could be attributed to the loss of β-adrenergic stimulation on contraction, Ca2+ transients, ICaL, and SR Ca2+ content in DTG myocytes; and 2) E-C coupling efficiency was significantly lower in DTG myocytes. In conclusion, increasing Cav1.2 activity by promoting its high-activity mode enhances cardiac contractility but decreases E-C coupling efficiency and the adrenergic reserve of the heart.

L-type calcium channel; β2a subunit; ventricular myocyte; β-adrenergic response

THE L-TYPE Ca2+ channel (LTCC or Cav1.2) is a high voltage-gated, long-lasting Ca2+ channel that is responsible for triggering Ca2+ release from the sarcoplasmic reticulum (SR) via a Ca2+-induced Ca2+ release process and for maintaining the plateau phase of the action potential in the heart (4). It consists of multiple subunits, with the α1C-subunit as the pore-forming

subunit and β- and αδ/δ-subunits as accessory subunits. The accessory subunits do not conduct Ca2+ but regulate the expression, trafficking, and functional properties of the α1C-subunit. The mRNA transcripts of all Cav1.2 subunits undergo alternative splicing, but the mechanism and significance of these alternatively spliced variants are not entirely clear. It has been noted that there is a switch of the expression of these splicing isoforms under pathological conditions such as heart failure (HF) (19, 36). Three Cavβ subunit genes have been identified, and the major β-isof orm in the heart is believed to be the β2-subunit (14, 19). The β3-subunit has multiple splicing variants: β2a, β2b, β2c, and β2h, among which β2b is the major splicing variant in human (19) and rodent (35) hearts and β2a is the variant with most significant modulation of α1C channel properties (10, 19, 25) by greatly enhancing its open probability.

Acutely after the imposition of cardiac stresses, the heart is hypercontractile due to the activation of the adrenergic system and other neurohumoral systems (1, 5, 6, 15, 18), causing a sustained increase in the activity of Cav1.2. If the stresses are sustained, then the heart remodels at molecular, cellular, and organ levels into a compensatory stage that is followed by cardiac failure. It has been reported that during the compensatory stage, cardiac contractility could be enhanced by increasing the sarcoplasmic reticulum (SR) Ca2+ load and the amplitude of Ca2+ sparks (26, 33). During the progression of HF, Cav1.2, along with all other Ca2+-handling proteins, remodels. Among these remodeling processes is a switch of the LTCC to a high-activity mode as well as activation and inactivation at more negative voltages (8, 9, 32). Both a high-phosphorylation state (8) and the change of the composition of multimeric Cav1.2 channels have been proposed for this high-activity mode. A splicing variant of the β2-subunit, β2a, is able to produce sustained LTCC Ca2+ influx (10, 25, 35) with the characteristics associated with HF, i.e., activation and inactivation at more negative voltages, enhanced LTCC activities at the single channel level (19, 25, 32, 35), and the loss of response to adrenergic stimulation (25). In accordance, β2a-subunit expression has been shown to be increased in failing human hearts (20). Our previous study (27) showed that when the β2a-subunit is overexpressed in the heart at a low level (3.5-fold increase), Ca2+ influx through the LTCC is increased and the heart develops moderate hypertrophy with interstitial fibrosis at the age of ~4 mo and cardiac dysfunction at the age of 12 mo. If β2a-subunit overexpression is further increased to a higher level, then the heart fails at the age of ~4 mo. In vitro,
\(\beta_{2a}\)-subunit overexpression in feline myocytes in primary culture induces myocyte apoptosis (10). Most recently, a mouse line overexpressing the \(\beta_{2a}\)-subunit was developed independently by another research group and showed subtle changes of LTCC current (\(I_{\text{Ca,L}}\)) without a significant change of cardiac contractility (3). However, to date, there is no detailed study on the effects of enhanced Cav1.2 activities on excitation-contraction (E-C) coupling and adrenergic responsiveness of the heart and its cardiac myocytes.

This study explored the impact of enhancing cardiac Cav1.2 activity on cardiac and myocyte contractility and their \(\beta\)-adrenergic regulation with a double transgenic (DTG) mouse line overexpressing the \(\beta_{2a}\)-subunit. We studied cardiac [with echocardiography and invasive intra-left ventricular (LV) pressure measurements] and myocyte (with electrophysiology and Ca\(^{2+}\) imaging) contractility and their regulation by an adrenergic agonist [isoproterenol (Iso)] in detail and examined E-C coupling efficiency in DTG myocytes. We discovered that cardiac and myocyte contractility are significantly enhanced in DTG mice at baseline, but they have reduced or even compromised responsiveness to \(\beta\)-adrenergic stimulation. E-C coupling efficiency was significantly reduced in DTG myocytes.

**MATERIALS AND METHODS**

*Generation of a DTG mouse line overexpressing the \(\beta_{2a}\)-subunit.* A transgenic mouse line overexpressing the \(\beta_{2a}\)-subunit was established with the inducible (controlled by doxycycline), bitransgenic, and cardiac-specific (\(\alpha\)-myosin heavy chain promoter) system (27, 31). DTG mice were used at the age of 4 mo, when \(\beta_{2a}\) gene expression had been fully induced, and non-DTG littermates were used as control (CTR) mice. Animal experiments were approved by the Institutional Animal Care and Use Committee of Temple University and conformed with the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Pub. No. 85-23, Revised 1996).

*Ventricular myocyte isolation.* Adult mouse myocyte isolation was done as previously described (27, 37). Myocytes from one DTG mouse and one control mouse were isolated on the same day and used in an alternated way for cellular measurements. Percentages of rod-shaped, Ca\(^{2+}\)-tolerant, live mouse ventricular myocytes (VMs) were \ (>70\%.

**Ca\(^{2+}\) current, \(\text{Ca}^{2+}\) transients, and contraction measurements.** Whole cell Ca\(^{2+}\) current (\(I_{\text{Ca,L}}\)) was measured in Na\(^{+}\)- and K\(^{+}\)-free solutions at 35°C using techniques previously described in detail (8). Myocytes were studied in a chamber mounted on an inverted microscope (Nikon) and were initially perfused with normal Tyrode salt solution containing (in mmol/l) 150 NaCl, 5.4 KCl, 1 CaCl\(_2\), 1.2 MgCl\(_2\), 10 glucose, 2 sodium pyruvate, and 5 HEPES (pH7.4) at 35°C. Low-resistance (2–5 M\(\Omega\)) patch pipettes filled with a solution containing (in mmol/l) 130 Cs-aspartate, 10 N-methyl-d-glutamate (NMDG), 20 tetraethylammonium (TEA)-Cl, 2.5 Tris-ATP, 0.05 Tris-GTP, 1 MgCl\(_2\), and 10 EGTA (pH 7.2) were used in whole cell voltage-clamp experiments. All myocytes were dialyzed with this pipette filling solution and perfused with normal physiological salt solution for 10 min before experiments were initiated. After the initial diastolic period, the perfusate was switched to a Na\(^{+}\)- and K\(^{+}\)-free bath solution containing (in mmol/l) 150 NMDG, 2 CaCl\(_2\), 5.4 CsCl, 1.2 MgCl\(_2\), 10 glucose, 5 HEPES, and 2 4-aminopyridine (pH 7.4). All experiments were performed in Na\(^{+}\)- and K\(^{+}\)-free (in and out) solutions so that Ca\(^{2+}\) currents were measured without contamination from overlying ionic currents. The junction potential was not corrected and was <10 mV. Cell capacitance was measured using −5-mV hyperpolarizing test steps from the holding potential (\(V_h\)). Membrane potentials were controlled with an Axopatch 2B voltage-clamp amplifier using pCLAMP10 (Molecular Devices) software, and data were acquired with a Digidata 1440 analog-to-digital converter (Molecular Devices). Data were analyzed with CLAMPfit (Molecular Devices) offline and presented with GraphPad Prism 5.0 (La Jolla, CA). Only myocytes with minimal (\(<10\%) rundown of \(I_{\text{Ca,L}}\) were included in the data sets. Current–voltage (\(I-V\)) relationships [−70 to 70 mV in 10-mV increments from \(V_h = -70 \text{ mV}\) of \(I_{\text{Ca,L}}\) were determined before and after the application of Iso (1 \(\mu\)M, Sigma, St. Louis, MO). Conductance–voltage (\(G-V\)) relationships were derived as previously described (8). Slow and fast time constants of \(I_{\text{Ca,L}}\) decay (\(\tau_s\) and \(\tau_f\), respectively) were determined by fitting the raw currents from the peak to 350 ms after the peak with the following double-exponential equation:

\[
\begin{align*}
I(t) &= A_1 \exp(-t/\tau_s) + A_2 \exp(-t/\tau_f) + A_0,
\end{align*}
\]

where \(A_1, A_2,\) and \(A_0\) are the amplitudes of \(I_{\text{Ca,L}}\) decay attributable to the fast, slow, and window components and \(t\) is time, as previously described (8). To determine the available channels on the sarcoplasmic membrane, charge movements (\(Q_{\text{on}}\) and \(Q_{\text{off}}\)) when VMs were depolarized to various test potentials (−30 to 60 mV) in 10-mV increments from \(V_h = -40 \text{ mV}\) and repolarized to \(V_h = -40 \text{ mV}\) were measured as described by He et al. (17) with an Axopatch 200B amplifier (Molecular Devices).

Cytoplasmic free Ca\(^{2+}\) concentrations (\([\text{Ca}^{2+}]_c\)) and Ca\(^{2+}\) transients were measured with a fluorescent Ca\(^{2+}\) indicator (Fluo 4-AM) in normal Tyrode solution containing 1 mM Ca\(^{2+}\) at 35°C as previously described (2). Our preliminary measurement with indo-1 indicated there were no significant differences in diastolic Ca\(^{2+}\) at a pacing frequency of 0.5 Hz. VMs were stimulated at 0.5 Hz, and [Ca\(^{2+}\)] (emitted fluorescence at 525 ± 10 nm) and cell length changes were recorded with an edge detector simultaneously. Once a steady state had been reached, 5 \(\times\) 10\(^{-5}\) M Iso was applied through the perfusion solution. Once a stable effect of Iso had been achieved, at least 20 continuous contractions and Ca\(^{2+}\) transients were recorded and averaged for analysis. Ca\(^{2+}\) transients were fit with a single-exponential decay function to determine the decay rate.

To measure SR Ca\(^{2+}\) content, myocytes were paced at 0.5 Hz for 10 consecutive contractions, and 10 mM caffeine was then rapidly applied via a glass pipette close to the myocyte with a Pico spritzer (29). Since caffeine cannot be repetitively applied on the same cell, the SR Ca\(^{2+}\) content after Iso was measured by a caffeine spritz after stable effects of Iso on myocyte contraction and Ca\(^{2+}\) transients had been observed. The decay of caffeine-induced Ca\(^{2+}\) transients was fit with a single-exponential function, and time constant (\(\tau\)) values indicate Na\(^{+}/\text{Ca}^{2+}\) exchange activity.

In vivo cardiac function evaluation with echocardiography. As previously described (24), in vivo \(\beta\)-adrenergic responsiveness was evaluated with echocardiography. Echocardiography was performed with a VisualSonics Vevo 770 machine, which was specifically designed for mice and rats. Mice were anesthetized with 2% isoflurane initially and then with 1% isoflurane during the echocardiography procedure to maintain their heart rates (HRs) between 400 and 450 beats/min. Hearts were viewed in the short axis between the two papillary muscles and analyzed in M-mode before and after the application of Iso (2 \(\mu\)g/g body wt ip). After the Iso injection, M-mode images were recorded every 1 min for 15 s for Iso effect determination. The parameters measured included end-diastolic diameter, end-systolic diameter, posterior wall thickness, septal wall thickness, ejection fraction (EF), HR, and fractional shortening (FS).

In vivo intra-LV pressure measurements. Intra-LV pressure was measured with a 1.4-Fr Millar pressure catheter (SPR-671, Millar Instruments, Houston, TX) connected to an AD Instruments PowerLab 16/30 (ADInstruments, Colorado Springs, CO) with LabChart Pro 6.0 software. In short, after mice had been anesthetized with ketamine-xylazine (50 and 2.5 mg/kg body wt ip, respectively) to maintain HRs in the 400–450 beats/min range, a midline neck incision was made, and the right carotid artery was exposed. The pressure catheter was inserted into the LV through a small incision on the right carotid artery and then advanced through the aortic valves into the LV. The catheter was carefully adjusted to avoid direct
contact with the ventricular wall so that smooth intra-LV pressure traces could be clearly recorded. Five minutes of baseline pressure were recorded, and Iso (2 mg/kg body wt) was then injected intraperitoneally. Intra-LV blood pressure was continuously recorded to monitor the Iso effect. Ten minutes of recording were made after the Iso injection. Data were analyzed offline with the blood pressure module in the LabChart6.0 software.

E-C coupling efficiency evaluation. E-C coupling efficiency in both CTR and DTG myocytes was evaluated by simultaneous recording of \( I_{\text{Ca,L}} \) \( [\text{Ca}^{2+}]_i \), and contraction with the whole cell voltage-clamp technique. The perfusion solution was normal physiological Tyrode solution, and the pipette solution was composed of the following (in mmol/l): 120 Cs-aspartate, 10 NMDG, 2.5 Tris-ATP, 0.05 Tris-GTP, 1.2 MgCl2, and 5.4 KCl (pH 7.2 with CsOH). Na+ current was eliminated by holding the cell at −40 mV (after junction potential correction), and K+ currents were suppressed by providing low intracellular K+ \( (5.4 \text{ mM}) \), extracellular Cs+, and intracellular TEA+. Since E-C coupling efficiency is profoundly affected by the SR loading condition, the E-C coupling process was studied in myocytes with matched SR Ca\(^{2+}\) content. For CTR myocytes, 10 consecutive square depolarization pulses \( \Delta t = +10 \text{ mV} \) for 100 ms at 1 Hz were applied to maintain the normal SR load before each test voltage (in 10-mV increments for 200 ms). For DTG myocytes, which have high SR Ca\(^{2+}\) content under normally paced conditions, 10 consecutive pulses of 5-ms depolarization \( \Delta t = +10 \text{ mV} \) were applied to decrease the SR load before each test voltage (in 10-mV increments for 200 ms). After the whole process was finished, SR Ca\(^{2+}\) content was measured. Only cells with comparable SR Ca\(^{2+}\) load were used for data analysis. The E-C coupling gain was calculated by dividing the amplitude of the Ca\(^{2+}\) transient by the amplitude of \( I_{\text{Ca,L}} \) at each voltage.

**Statistics.** Data are reported as means ± SE. When appropriate, paired and unpaired t-tests, ANOVA, or ANOVA for repeated measures were used to detect significance with SAS 9.0 (SAS Institute, Cary, NC). \( P \) values of \( \leq 0.05 \) were considered significant. In this report, \( n \) represents the number of isolated cells used and \( N \) represents the number of animals used.

**RESULTS**

Myocytes from DTG mice are hypercontractile with increased \( I_{\text{Ca,L}} \) and SR \( \text{Ca}^{2+} \) content. The \( \beta_2\alpha\)-subunit is an accessory subunit of the LTCC that enhances the trafficking of the \( \alpha_{1c}\)-subunit to the sarcolemmal membrane and increase the open probability of the \( \alpha_{1c}\)-subunit (12). It also shifts the activation of Cav1.2 to more negative membrane potentials. In agreement with these findings, maximal \( I_{\text{Ca,L}} \) density was significantly larger in DTG myocytes (23.7 ± 2.4 pA/pF, \( n = 8 \)) than in CTR VMs (11.8 ± 1.7 pA/pF, \( n = 8 \)). The \( I-V \) relationship peaked at −10 mV in DTG myocytes and at a more positive voltage (0 mV) in CTR myocytes (Fig. 1A). Further analysis by fitting G-V relationships with the Boltzmann equation revealed that the half-maximum activation voltage in DTG myocytes (−28.1 ± 2.8 mV) was significantly more negative than in CTR myocytes (−18.3 ± 2.1 mV, \( P < 0.05 \) by t-test). The decay of \( I_{\text{Ca,L}} \) in DTG and CTR VMs was not different in that \( \tau_1 \) (Fig. 1B) and \( \tau_2 \) (Fig. 1C) at the test potentials from −40 to 40 mV were not significantly different between these two groups. These results verified that the \( \beta_2\alpha\)-subunit was overexpressed and enhanced \( I_{\text{Ca,L}} \) in our transgenic mice. It has been reported that the \( \beta_2\alpha\)-subunit increases \( I_{\text{Ca,L}} \) by enhancing \( \alpha_{1c}\)-subunit trafficking to the surface membrane and augmenting the open probability of the \( \alpha_{1c}\)-subunit (14). Our previous study (27) using Western blot analysis did not detect an expression difference in the \( \beta_2\alpha\)-subunit between DTG and CTR myocytes. However, that study did not specifically determine the amount of the \( \alpha_{1c}\)-subunit on the surface mem-
brane. In this study, charge movement measurements were done to evaluate total available Cav1.2 on the surface membrane. Our data showed an insignificant increase of both $Q_{on}$ and $Q_{off}$ (Fig. 1D), indicating that the major mechanism for increasing $I_{Ca_{L}}$, in DTG myocytes was by increasing the open probability of the LTCC by the $\beta_{2\alpha}$-subunit.

Increasing Ca$^{2+}$ influx is capable of enhancing cardiac myocyte contractility because augmented $I_{Ca_{L}}$ serves as a greater trigger for E-C coupling and also increases SR Ca$^{2+}$ content (4). Consistent with these effects, FS was significantly greater in DTG myocytes (9.25 ± 0.74%, $n = 28$, $N = 4$) than in CTR myocytes (4.94 ± 0.64%, $n = 28$, $N = 4$; Fig. 2A). The peak Ca$^{2+}$ transient (fluor-4, F/F$0$) evoked by 0.5-Hz electrical pacing was significantly larger in DTG myocytes (DTG vs. CTR VMs: 3.37 ± 1.58, $n = 28$, $N = 4$, vs. 2.45 ± 0.08, $n = 28$, $N = 4$; Fig. 2, B and C). There was enhanced SR Ca$^{2+}$ content [peak of the caffeine-induced Ca$^{2+}$ transient with fluo-4 in DTG myocytes (F/F$0$): 4.7 ± 0.4 in DTG VMs vs. 3.2 ± 0.3 in CTR VMs: Fig. 2, B and C].

**DTG hearts have enhanced cardiac contractility.** We further determined in vivo cardiac function in CTR and DTG mice at the age of 4 mo, when the $\beta_{2\alpha}$ transgene was fully induced with echocardiography and invasive intra-LV pressure measurements. DTG hearts had significantly greater FS (CTR vs. DTG: 36.2 ± 1.0%, $N = 12$, vs. 42.6 ± 1.2%, $N = 7$, $P < 0.05$; Fig. 3C) and EF (CTR vs. DTG: 61.0 ± 2.0%, $N = 12$, vs. 71.8 ± 2.3%, $N = 7$, $P < 0.01$; Fig. 3D) when the HR under anesthesia in both groups was controlled at similar levels (CTR: 455 ± 15 beats/min and DTG: 477 ± 17 beats/min; Fig. 3B). Invasive intra-LV hemodynamic measurements showed that the maximum pressure of pressure development (dP/dt) and decay (−dP/dt) were significantly faster (Fig. 3, E and G) in DTG mice (dP/dt: 9,604.7 ± 961.8 mmHg/s and −dP/dt: −6,724.4 ± 442.3, $N = 6$) than in CTR mice (dP/dt: 6,490.9 ± 669.0 mmHg/s and −dP/dt: −4,620.1 ± 141.7, $N = 11$) in anesthetized mice with similar HRs (CTR: 462 ± 27 beats/min vs. DTG: 455 ± 17 beats/min; Fig. 3F). These data show that DTG hearts and myocytes are hypercontractile.

**Hypercontractile DTG hearts lose $\beta$-adrenergic responsiveness.** We further explored if hypercontractile DTG hearts had normal $\beta$-adrenergic responsiveness with both echocardiography and hemodynamic measurements. A maximally effective dose of Iso (2 $\mu$g/g body wt) was injected into mice intraperitoneally after the basal echocardiographic measurements had been done as previously described (24). At 4 min after the injection, all studied mice reached a stable and maximal response to Iso. HRs were increased to the same extent in CTR and DTG hearts (CTR: from 462 ± 27 to 558 ± 13 beats/min vs. DTG: from 455 ± 17 to 565 ± 4 beats/min; Fig. 4A). FS (CTR: from 36.2 ± 1.0% to 57.4 ± 2.4%, $N = 12$, vs. DTG: from 42.6 ± 1.2% to

---

**Fig. 2.** DTG myocytes ($n = 28$, $N = 4$) have increased Ca$^{2+}$ transients, sarcoplasmic reticulum (SR) Ca$^{2+}$ content, and faster decay of caffeine-induced Ca$^{2+}$ transients than CTR ($n = 28$, $N = 4$) myocytes. A: myocyte fractional shortening (FS) was significantly greater in DTG myocytes. Inset, example of myocyte contraction and relengthening normalized to resting length. B: examples of Ca$^{2+}$ transients elicited by 0.5-Hz electrical field stimulation (the first four traces) followed by Ca$^{2+}$ transients induced by a caffeine spritz (the fifth large transient; the peak indicates SR Ca$^{2+}$ content) in one CTR myocyte and one DTG myocyte loaded with fluo-4, a fluorescent Ca$^{2+}$ indicator. C: average amplitudes of pacing- and caffeine-induced Ca$^{2+}$ transients in CTR and DTG myocytes. Significantly greater pacing- and caffeine-induced Ca$^{2+}$ transient amplitudes were observed in DTG myocytes. D: decay of Ca$^{2+}$ transients assessed by the time constant (τ) of the fitting of the Ca$^{2+}$ transient decay with a single-exponential function was not different between DTG (89.6 ± 15.8 ms, $n = 28$, $N = 4$) and CTR (88.3 ± 6.2 ms, $n = 28$, $N = 4$) VMs. [Ca$^{2+}$]cystolic free Ca$^{2+}$ concentration. E: there were significantly smaller $\tau$ values of the fitting of the decay of caffeine-induced Ca$^{2+}$ transients in DTG (153.0 ± 222.7 ms, $n = 28$, $N = 4$) and CTR (2,249.0 ± 121.8 ms, $n = 28$, $N = 4$) VMs. Two-tailed Student’s $t$-tests were used for significance detection in A–E.

**AJP-Heart Circ Physiol** • VOL 299 • AUGUST 2010 • www.ajpheart.org
DTG myocytes lose responsiveness to β-adrenergic stimulation. DTG hearts responded abnormally to adrenergic agonists. To determine the cellular mechanism responsible for this phenomenon, myocytes were exposed to Iso. Iso enhanced myocyte FS and intracellular Ca²⁺ transients in CTR myocytes but not in DTG myocytes (Fig. 6, A and B). This could be due to the fact that the contractility of DTG myocytes was already near the maximal level. Myocyte contraction and intracellular Ca²⁺ transient amplitudes are dictated by the Ca²⁺ available for release in the SR (SR Ca²⁺ load) and the size of the trigger (I_{Ca,L}) (4). The SR Ca²⁺ content estimated by the peak amplitude of caffeine-induced Ca²⁺ transients was enhanced by Iso in CTR myocytes but not in DTG myocytes (Fig. 6E), nor did Iso increase I_{Ca,L} in DTG myocytes (Fig. 6D). These results suggest that DTG myocytes have near-maximal contractility under basal conditions and that Iso fails to increase their contractility further because of its failure to increase I_{Ca,L} and SR Ca²⁺ load.

Reduced E-C coupling efficiency in DTG myocytes. Since DTG myocytes have enhanced contractility due to both increased I_{Ca,L} and SR Ca²⁺ content, it would be interesting to study the E-C coupling efficiency. E-C coupling is greatly influenced by the loading condition of the SR because high SR luminal Ca²⁺ enhances the opening probability of the ryanodine receptor (4). To make a fair comparison of E-C coupling efficiency between DTG and CTR myocytes, SR contents of the myocytes were controlled and only myocytes having similar SR Ca²⁺ content were analyzed. Five DTG myocytes and five CTR myocytes with similar SR Ca²⁺ content (Fig. 7E) were selected from eighteen DTG and seventeen CTR myocytes, respectively. Figure 7A shows examples of simultaneous recordings of I_{Ca,L}, myocyte contraction, and Ca²⁺ transients in a CTR myocyte and a DTG myocyte having similar SR Ca²⁺ content. The Ca²⁺ transients induced by depolarizing the cell to different test potentials (−30 to −40 mV) in DTG...
myocytes were slightly but insignificantly higher than those in CTR myocytes (Fig. 7C) and appeared longer in duration, possibly due to secondary Ca\(^{2+}\) release induced by the increased \(I_{\text{Ca,L}}\) window current in DTG myocytes. \(I_{\text{Ca,L}}\) was significantly greater at test potentials from -20 to -40 mV in DTG myocytes than in CTR myocytes (Fig. 7B). The E-C coupling gain, measured by dividing the amplitude of the Ca\(^{2+}\) transient by the amplitude of \(I_{\text{Ca,L}}\) at the same test voltage, was significantly greater in CTR myocytes than in DTG myocytes at test voltages from -20 to 40 mV (Fig. 7D), suggesting that Ca\(^{2+}\) influx into the junctional space between the T-tubules and SR of DTG myocytes is excessive and beyond what is needed to initiate full SR Ca\(^{2+}\) release.

**DISCUSSION**

HF is associated with cardiac remodeling at the organ, myocyte, and molecular levels. Myocyte remodeling involves the switch of Cav1.2 to a highly active mode (8, 9, 32) due to either hyperphosphorylation (8, 9) or alterations of the molecular composition of this multimeric channel and the shift of Cav1.2 activation and inactivation to more negative voltages (8). The high-activity mode of Cav1.2 in failing myocytes is associated with the loss of its responsiveness of \(\beta\)-adrenergic stimulation (8). These features of Cav1.2 in failing cardiac myocytes can be mimicked by a splicing variant of the auxiliary \(\beta\)-subunit (\(\beta_2\)a-subunit) when coexpressed with the \(\alpha_{1C}\)-subunit (19, 25). The \(\beta_2\)a-subunit alters the \(\beta\)-adrenergic/PKA regulation of Cav1.2 (11, 25). In this study, we further explored the functional consequences of increases in Cav1.2 activity in cardiac myocytes in vivo and in vitro with a transgenic mouse model overexpressing the \(\beta_2\)a-subunit. We found that 1) \(\beta_2\)a-subunit overexpression in cardiac myocytes in transgenic mice induces a hypercontractile cardiac phenotype, which was caused by enhanced Ca\(^{2+}\) influx and increased SR Ca\(^{2+}\) content in the myocyte; 2) the enhanced cardiac contractility brought about by increased Ca\(^{2+}\) influx blunts or even reverses (negative inotropic effects) \(\beta\)-adrenergic responses of the heart and myocyte due to a loss of cardiac/myocyte contractility reserve and Ca\(^{2+}\) overload; and 3) E-C coupling efficiency is decreased in DTG myocytes.

These results have implications for the bases of reduced functional and adrenergic reserve in hearts undergoing remodeling and suggest that HF therapies to enhance cardiac contractility by...
manipulating myocyte $\text{Ca}^{2+}$ handling should be approached cautiously.

Does our $\beta_2\alpha$-subunit-overexpressing mouse model recapitulate the cardiac phenotype during HF development? Based on results reported in a previous study (27) and the present study, the mouse model overexpressing the $\beta_2\alpha$-subunit at a low level might simulate $\text{Ca}^{2+}$ handling during the compensated cardiac hypertrophy stage. The evidence to support this conclusion includes the following. First, when the heart is under stress, the sympathetic nervous system is activated, which increases LTCC activity. At the same time, the LTCC remodels into a high-activity mode either due to a composition change or hyperphosphorylation when the heart advances into HF (8, 9, 32). These characteristics of LTCCs in the heart under stress are well mimicked by the overexpression of the $\beta_2\alpha$-subunit (19, 25, 32, 35). It has been shown that the expression of $\beta_2\alpha$-subunit splicing variants, including the $\beta_2\alpha$-subunit, is increased in failing human hearts (20). We suspect that the

Fig. 5. Compromised Iso response of DTG hearts evaluated with intra-LV hemodynamic measurements. A: examples of intra-LV pressure recording, derived $dP/dt$ and HR before and after the injection of Iso (indicated by the arrow) in a CTR mouse. Please note the quick rise of the HR and corresponding increase in systolic blood pressure and $dP/dt$ after the Iso injection (peak 1), which was followed by a decreasing phase of systolic blood pressure and maximum $dP/dt$. Then, at ~2.5 min after the Iso injection, systolic blood pressure and maximum $dP/dt$ were increased to stable levels again (peak 2). B: examples of intra-LV pressure recording, derived $dP/dt$, and HR before and after the injection of Iso (indicated by the arrow) in a DTG mouse. Unlike in the CTR mouse, Iso did not increase systolic pressure and $dP/dt$ but rather decreased them, although the HR was increased. C: Iso increased HRs to similar levels (from baseline to peak 1 to peak 2: from 462 ± 27 to 607 ± 26 to 609 ± 22 beats/min in CTR mice and from 465 ± 17 to 597 ± 17 to 601 ± 29 in DTG mice) in both CTR and DTG mice. D: at baseline, the systolic LV pressure was slightly but not significantly higher in DTG mice. Iso caused a double-phase increase in systolic pressure in CTR mice (from 82.8 ± 5.3 to 102.5 ± 9.9 to 107.9 ± 13.0 mmHg) but a continuous decrease in DTG mice (from 91.7 ± 9.5 to 77.0 ± 5.4 to 72.8 ± 8.0 mmHg). E: Iso did not significantly change LV end-diastolic pressure (EDP) in CTR mice (from 3.19 ± 2.41 to 2.15 ± 3.3 to 3.64 ± 1.71 mmHg) but increased it in DTG mice (from 3.82 ± 2.08 to 5.86 ± 2.46 to 10.32 ± 2.48 mmHg). F: Iso increased developed LV pressure (systolic pressure − EDP) in CTR mice (from 83.9 ± 4.5 to 104.6 ± 8.0 to 108.1 ± 7.8 mmHg) but decreased it in DTG mice (from 92.3 ± 7.4 to 74.6 ± 5.3 to 69.3 ± 11.9 mmHg). G: maximum $dP/dt$ was enhanced by Iso in CTR mice (from 6,490.0 ± 669.6 to 11,967.2 ± 836.6 to 13,675.4 ± 1,696.7 mmHg/s) but increased in DTG mice (from 9,078.5 ± 1,084.7 to 5,914.2 ± 1,115.6 to 6,650.9 ± 1,045.6 mmHg/s). H: Iso increased the maximum LV pressure decay rate ($-dP/dt$) in CTR mice (−4,620.1 ± 141.7 to −6,730.9 ± 279.1 to −7,226.5 ± 395.1 mmHg/s) but not in DTG mice (−6,724.4 ± 442.3 to −4,912.8 ± 343.3 to −4,764.5 ± 570.8 mmHg/s). Two-way ANOVA with repeated measurements and post hoc t-tests were used for significance detection in C–H.
expression of the β2a-subunit is already elevated in compensated hypertrophic hearts. Second, we (27) have previously shown that the mice used in this study overexpress the β2a-subunit at a low level (3.5-fold) and develop moderate cardiac hypertrophy, interstitial fibrosis, and enhanced cardiac myocyte death at the age of 4 mo. This mouse line eventually develops HF at the age of 12 mo. Third, in this study, we showed that β2a-subunit-overexpressing DTG mice have cardiac hyperfunction with depressed β-adrenergic responses, which very likely simulates the compensated cardiac hypertrophy stage. Cardiac hyperfunction after cardiac stress induction has been reported (1, 5, 6, 15, 18). At these early compensated stages, the myocardium (26) and myocytes (7, 26) can be hypercontractile. In a postmyocardial infarction mouse HF model, myocytes isolated from both early and late stages of HF had higher than normal I_{Ca,L} and SR Ca^{2+} content (26).

Finally, our results show decreased E-C coupling gain in DTG myocytes and are in good agreement with the decreased E-C coupling gain in hypertrophied myocytes as well (16). We are not sure if this is due to the structural or functional alterations of the myocytes or simply an oversaturated Ca^{2+}-induced Ca^{2+} release trigger (I_{Ca,L}) (34), which we view as the most likely reason for our findings.

Recently, there has been a report (3) showing that in a β2a-subunit-overexpressing transgenic mouse model independently developed by the Beetz et al. group, I_{Ca,L} was moderately increased with a significant leftward shift of the voltage dependency of I_{Ca,L} activation and mode 2 activities. When this mouse line was crossbred with an α1c-subunit-overexpressing mouse line, I_{Ca,L} was increased by 61.2%, but no cardiac functional enhancement was observed, and dP/dt was actually decreased. The authors suggested that these phenotypes represent what have been observed in failing hearts, assuming that the heart in the transgenic mice went into HF in 4 wk. It is unclear why we found such different results in our β2a-subunit transgenic mice, but it could be due to the fact that our model had a higher basal I_{Ca,L}.

Our experiments show that in hypercontractile DTG hearts, when the HR was raised to close to or above normal, end-diastolic blood pressure was significantly increased, whereas developed pressure, maximum dP/dt, and minimum −dP/dt were significantly reduced, which were even below the basal contractility in CTR animals. This could be due to SR Ca^{2+} overload and a resultant elevation in diastolic [Ca^{2+}], in DTG myocytes when DTG hearts beat at higher HRs and the impact of interstitial fibrosis (27) on cardiac function. Alternatively, the change of the morphology (e.g., cardiac hypertrophy) of the heart after β2a-subunit overexpression causes abnormal systolic anterior motion of the mitral valve [abnormal systolic anterior motion causes negative ISO inotropic effects on the heart (23)], which needs to be further explored. These results unveil a potential mechanism for heart disease progression: at first, myocytes remodel and develop hypercontractility to support the increased cardiac demand. However, enhancing myocyte contractility by enhancing Ca^{2+} handling has an associated detrimental effect on diastolic and systolic function, which is likely caused by SR Ca^{2+} overload and leak. These changes may underlie Ca^{2+}-dependent molecular and cellular remodeling, such as in hypertrophy (7, 27). Long-term myocyte Ca^{2+} overload can lead to myocyte death and fibrosis (10, 27), which increases the contractile demand on surviving myocytes, which leads to a vicious cycle and ultimately causes HF.

Is enhancing Ca^{2+} handling a way of HF prevention and treatment? In the early days of searching for HF treatments, raising myocyte contractility by enhancing Ca^{2+} influx with Cav1.2 agonists or β-adrenergic agonists was explored (21, 28). These studies (21, 28) have shown increased mortality in animals and patients receiving such treatments. Our study offers some clues to explain these adverse effects of increasing Ca^{2+} influx. The increased Ca^{2+} influx, combined with decreased sarco(endo)plasmic reticulum Ca^{2+}-ATPase (SERCA) expression, in failing myocytes leads to further...
diastolic Ca\(^{2+}\) overload, worsening the loss of myocytes and arrhythmias. On the other hand, if SERCA activity is enhanced by either overexpressing SERCA (13, 22, 30) or knockout of phospholamban, diastolic Ca\(^{2+}\) overload can be prevented, and the subsequent myocyte death and fibrosis can be reduced. More recently, SERCA has been used in preclinical and clinical trials to normalize Ca\(^{2+}\) handling in the failing myocytes (13, 22, 30). The results are promising and suggest that correcting Ca\(^{2+}\) handling defects downstream of Ca\(^{2+}\) influx may be beneficial to HF patients.

**Conclusions.** In conclusion, we show that myocytes with increased Ca\(^{2+}\) influx are hypercontractile but have reduced \(\beta\)-adrenergic and contractility reserve and are prone to both diastolic and systolic dysfunctions.

**GRANTS**

This work was supported by National Heart, Lung, and Blood Institute Grants HL-089312 (to S. R. Houser) and HL-088243 (to X. Chen) and by American Heart Association Scientist Development Grant 0730347N (to X. Chen).


