Mechanism of Enhanced Cardiac Function in Mice with Hypertrophy Induced by Overexpressed Akt*

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Transgenic mice with cardiac-specific overexpression of active Akt (TG) not only exhibit hypertrophy but also show enhanced left ventricular (LV) function. In 3-4-month-old TG, heart/body weight was increased by 60% and LV ejection fraction was elevated (84 ± 2%, p < 0.01) compared with nontransgenic littermates (wild type (WT)) (73 ± 1%). An increase in isolated ventricular myocyte contractile function (% contraction) in TG compared with WT (6.1 ± 0.2 versus 3.5 ± 0.2%, p < 0.01) was associated with increased Fura-2 Ca²⁺ transients (396 ± 50 versus 250 ± 24 nmol/liter, p < 0.05). The rate of relaxation (dI/dt) was also enhanced in TG (214 ± 15 versus 98 ± 18 µm/s, p < 0.01). L-type Ca²⁺ current (I_Ca(L)) density was increased in TG compared with WT (∼9.0 ± 0.3 versus 7.2 ± 0.3 pA/pF, p < 0.01). Sarcoplasmic reticulum Ca²⁺-ATPase 2a (SERCA2a) protein levels were increased (p < 0.05) by 6.6-fold in TG, which could be recapitated in vitro by adenovirus-mediated overexpression of Akt in cultured adult ventricular myocytes. Conversely, inhibiting SERCA with either ryanodine or thapsigargin affected myocyte contraction and relaxation and Ca²⁺ channel kinetics more in TG than in WT. Thus, myocytes from mice with overexpressed Akt demonstrated enhanced contractility and relaxation, Fura-2 Ca²⁺ transients, and Ca²⁺ channel currents. Furthermore, increased protein expression of SERCA2a plays an important role in mediating enhanced LV function by Akt. Up-regulation of SERCA2a expression and enhanced LV myocyte contraction and relaxation in Akt-induced hypertrophy is opposite to the down-regulation of SERCA2a and reduced contractile function observed in many other forms of LV hypertrophy.

PKB/Akt, a 57-kDa protein, is a serine-threonine kinase and exists as three isoforms, α, β, and γ (Akt1, Akt2, and Akt3, respectively) (1). Akt is activated by various growth stimuli including insulin, platelet-derived growth factor, insulin-like growth factor-1, and agonists for the β-adrenergic receptor, as well as stresses, such as shear stress (2). Activated Akt phosphorylates various downstream targets, thereby modulating diverse cellular functions including cell survival and apoptosis, protein synthesis, and glycogen metabolism (1–6). We have shown that Akt mediates β-adrenergic receptor-induced cardiac hypertrophy by negatively regulating glycogen synthase kinase-3β in neonatal rat cardiac myocytes (7, 8). Recent reports have shown that cardiac-specific overexpression of active mutants of Akt induces hypertrophy in transgenic mice in vivo (9–11). Akt activation also exerts a cardioprotective effect by inhibiting cardiomyocyte death during ischemia/reperfusion (12–16). The cardioprotection is associated with reduced infarct size as well as preserved function of surviving myocardium (14).

Besides these well-characterized functions of Akt, transgenic mice with cardiac-specific overexpression of constitutively active Akt have increased base-line contractility, namely an elevated LV dP/dt max (11). However, the cellular mechanism responsible for increased myocardial function by Akt activation remains unknown. In this study, we examined the correlation of in vivo cardiac function with in vitro intrinsic myocyte contraction and relaxation. To determine the cellular mechanism of the enhanced cardiac function in TG, we examined L-type Ca²⁺ channel function and Ca²⁺-handling proteins, including sarcoplasmic reticulum (SR) Ca²⁺ ATPase 2a (SERCA2a), phospholamban (PLB), calsequestrin, the α-subunit of the L-type Ca²⁺ channel (α1c), and Na+/Ca²⁺ exchanger (NCX), as well as myocardial ryanodine receptor binding. Our results suggest that activation of Akt enhances Ca²⁺ transients and facilitates both contraction and relaxation in isolated cardiac myocytes, which is associated with enhanced Ca²⁺ influx and increased protein levels of SERCA2a. These features indicate a novel function of Akt in the mouse heart.

**EXPERIMENTAL PROCEDURES**

**Transgenic Mice—**The generation of transgenic mice with cardiac-specific overexpression of constitutively active Akt (E40K Akt) (TG) has been described (11). We have also generated transgenic mice with **

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1 The abbreviations used are: LV, left ventricular; TG, transgenic mice with cardiac-specific overexpression of active Akt; SR, sarcoplasmic reticulum; SERCA, sarcoplasmic reticulum Ca²⁺ ATPase; PLB, phospholamban; NCX, Na⁺/Ca²⁺ exchanger; α1c, L-type Ca²⁺ channel; Ki, kinase inactive; WT, wild type mice.
cardiac-specific overexpression of kinase-inactive Akt (Tg-KI-Akt). Tg-KI-Akt did not exhibit an obviously abnormal cardiac phenotype, and cardiac function was apparently normal. In this study, 3–5-month-old TG, Tg-KI-Akt, and nontransgenic littermate mice (WT) were used. Animals used in this study were maintained in accordance with the Guide for the Care and Use of Laboratory Animals (37).

Echocardiography—Mice were anesthetized with an intraperitoneal injection of ketamine (0.065 mg/g), xepamazine (0.02 mg/g), and xylazine (0.013 mg/g). The procedure for echocardiography has been reported previously (17, 18).

Measurement of Contraction and Relaxation and Ca\(^{2+}\) Transients—Adult mouse ventricular cardiac myocytes were isolated as described previously (19). The external solution contained 1 mM CaCl\(_2\) in a total volume of 150 mmol/liter.

Whole-cell L-type Ca\(^{2+}\) currents and from the Na\(^+/\)Ca\(^{2+}\) exchanger.

Western Blot Analysis for SERCA2a, PLB, Calsequestrin, \(\alpha_c\), and NCX—Left ventricular tissue was obtained and immediately frozen in liquid nitrogen (–80°C). On the day of the experiment, tissue samples were homogenized in 0.75 M NaCl, 10 mM histidine (pH 7.5) with a one-ninth volume of 10 mM NaPO\(_4\), 10 glucose (1.0 g/ml) and 5 HEPES (pH 7.4). The heart was digested using collagenase 1 and 2, 75 units/ml each (Worthington) at 32 ± 2°C. All solutions were bubbled continuously with 95% O\(_2\) and 5% CO\(_2\) during digestion.

Myocytes were field-stimulated at 1 Hz, and contraction was measured using a video motion edge detector (VED103, Cresscent Electronics) as described previously (19). The external solution contained 1 mm Ca\(^{2+}\). The following contractile properties were calculated from length data: % contraction and the rate of shortening (–dL/dt). Relaxation properties (% dL/dt, the rate of relengthening, t\(_{70}\), the time for 70% relaxation) were also assessed. SR Ca\(^{2+}\) reuptake function was examined in response to thapsigargin (10–3, 10–4, and 10–5 M) in both WT and TG. Cells were loaded with 5 µM Pura-2 AM (Sigma), and Ca\(^{2+}\) transients were measured using a monochromatic dual beam spectrofluorometer (Photon Technology) (19).

Electrophysiology—Whole-cell L-type Ca\(^{2+}\) currents were measured using patch clamp techniques, as described previously (19). The external solution contained (mmol/liter): 2 CaCl\(_2\), 1 MgCl\(_2\), 135 tetraethyl ammonium chloride, 5 4-aminoypyridine, 10 glucose, and 5 HEPES (pH 7.5). The pipette solution contained (mmol/liter): 100 Ca-aspartate, 20 CaCl\(_2\), 1 MgCl\(_2\), 2 MgATP, 0.5 GTP, 5 EGTA, and 5 HEPES (pH 7.5). These solutions provided isolation of Ca\(^{2+}\) currents from other membrane currents and from the Na\(^+/Ca\(^{2+}\) exchanger.

Western Blot Analysis for SERCA2a, PLB, Calsequestrin, \(\alpha_c\), and NCX—Left ventricular tissue was obtained and immediately frozen in liquid nitrogen (–80°C). On the day of the experiment, tissue samples were homogenized in 0.75 mM NaCl, 10 mM histidine (pH 7.5) with protease, phosphatase, and kinase inhibitors. Equal amounts of protein were dissolved in 1% SDS, 100 mmol/liter Tris-HCl (pH 6.5), 10% glycerol, 0.05% bromphenol blue, 5% mercaptoethanol, separated by 12.5% SDS-PAGE, and transferred onto polyvinylidene difluoride membranes. Blots for SERCA2a and PLB were incubated for 30 min at room temperature with a 1:20,000 dilution of rabbit anti-SERCA2a polyclonal antibody (generous gift from Dr. Frank Wuytack, Leuven, Belgium) or a 1:20,000 mouse anti-PLB monoclonal Ab (Affinity BioReagents Inc., Golden, CO) in Tris-buffered saline containing 0.1% Tween 20 and 5% nonfat milk. Blots for calsequestrin were incubated overnight at 4°C with a 1:25,000 dilution of rabbit anti-calsequestrin calsequestrin polyclonal antibody (Upstate Biotechnology, Lake Placid, NY) in the same buffer. Blots for NCX were incubated overnight at 4°C with a 1:1,000 dilution of monoclonal anti-NCX (Swant) in Tris-buffered saline and 1% milk. Blots for \(\alpha_c\) were incubated overnight at 4°C with a 1:500 dilution of anti-\(\alpha_c\) antibody (Affinity BioReagents) in Tris-buffered saline and 1% milk. Intensities of the bands were evaluated by densitometric scanning using a Personal Densitometer SI with ImageQuant software (Amersham Biosciences) and normalized for protein loading.

Alkaline Phosphatase Treatment—Cell lysates (15 µg) were treated with a one-ninth volume of 10× alkaline phosphatase buffer (Promega, Madison, WI) at 30°C for 10 min and then treated with 30 units of calf intestine alkaline phosphatase at 30°C for 10 min. 4× SDS-PAGE loading buffer was added, and the samples were separated by 8% SDS-PAGE. Then, SERCA2a protein was processed for Western blotting as described above.

Rykydine Receptor Binding Studies—Rykydine receptor binding studies were conducted as described previously (20). Assays were performed using 6 concentrations of (H)ryanodine (1 to 30 nmol/liter) and 100 µg of membrane protein in HEPES buffer (with 0.3 mM BaCl\(_2\)) in a total volume of 150 µL. Unlabeled ryanodine (10 µmol/liter) was used to determine nonspecific binding. Incubation was at 37°C for 90 min. All assays were performed in triplicate and terminated by rapid filtration through Whatman GF/F filters washed with 4 mL of cold buffer (150 mmol/liter KCl, 10 mmol/liter Tris HCl, pH 7.4). Filters were

Cardiac sarcomere analysis was performed with the using a one-ninth volume of 10× alkaline phosphatase buffer (Promega, Madison, WI) at 30°C for 10 min and then treated with 30 units of calf intestine alkaline phosphatase at 30°C for 10 min. 4× SDS-PAGE loading buffer was added, and the samples were separated by 8% SDS-PAGE. Then, SERCA2a protein was processed for Western blotting as described above.

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### Table 1

**Left ventricular function measured by echocardiography**

| Function                                      | WT (n = 9) | TG (n = 6) |
|-----------------------------------------------|------------|------------|
| Heart rate (bpm)                              | 287 ± 16   | 318 ± 22   |
| EDD (mm)                                      | 3.6 ± 0.2  | 3.7 ± 0.1  |
| ESD (mm)                                      | 2.3 ± 0.1  | 2.0 ± 0.1* |
| EDWT (mm)                                     | 0.62 ± 0.03| 0.84 ± 0.06*|
| Ejection fraction (%)                         | 73 ± 1     | 84 ± 2*    |
| Fractional shortening (%)                     | 36 ± 7     | 47 ± 2*    |

* p < 0.05 versus WT. Values are mean ± S.E.

Fraction was significantly enhanced (p < 0.01) in TG (84 ± 2% compared with WT (p < 0.05). The LV ejection fraction was significantly enhanced (p < 0.01) in TG (84 ± 2%) compared with WT mice (73 ± 16%). These results suggest that TG have concentric hypertrophy with increased systolic function.

**Echocardiography**—Heart weight/body weight ratio was increased (p < 0.01) in TG (11.5 ± 1.4 mg/g) compared with WT (7.2 ± 0.4 mg/g). The results of echocardiographic measurements are summarized in Table I. Heart rate was similar between TG and WT. The end diastolic wall thickness was increased (p < 0.01) in TG (0.84 ± 0.06 mm) compared with WT (0.62 ± 0.03 mm). Although end diastolic dimensions were similar, end systolic dimensions were significantly reduced by 15% in TG compared with WT (p < 0.05). The LV ejection fraction was significantly enhanced (p < 0.01) in TG (84 ± 2%) compared with WT mice (73 ± 16%). These results suggest that TG have concentric hypertrophy with increased systolic function.

**Myocyte Contraction and Ca\(^{2+}\) Transients**—Fig. 1 shows representative contraction/relaxation and Ca\(^{2+}\) transient record.
Akt Induces Ca\(^{2+}\)-mediated Enhanced Myocyte Function

Akt overexpression is dependent upon the kinase activity of Akt. Akt Induces Ca\(^{2+}\) transients in adult ventricular cardiac myocytes isolated from WT and TG. As summarized in Table II, quantitative analyses indicated that percent contraction was significantly increased in TG myocytes compared with WT myocytes (6.1 ± 0.2 versus 3.5 ± 0.2%, p < 0.01), and the rate of contraction (−dL/dt) was also significantly increased in TG myocytes compared with WT myocytes (−284 ± 15 versus −147 ± 20 μm/s, p < 0.01). Relaxation function in TG myocytes was also enhanced compared with WT myocytes; the rate of relengthening (+dL/dt) was significantly faster, (214 ± 15 versus 98 ± 18 μm/s, p < 0.01) and the time for 70% relaxation was decreased in TG myocytes (48 ± 2 versus 67 ± 5 ms, p < 0.01). The effect of constitutively active Akt overexpression is dependent upon the kinase activity of Akt, because myocytes isolated from Tg-KI-Akt did not show enhanced contraction and relaxation function. These results suggest that increased myocardial contraction seen in TG hearts in vivo is also observed in individual cardiac myocytes isolated from TG.

To examine whether the enhanced contraction and relaxation in TG myocytes are caused by changes in intracellular Ca\(^{2+}\) levels, we measured Ca\(^{2+}\) transients. As shown in Fig. 1 and Table II, the amplitude of Ca\(^{2+}\) transients in TG myocytes was significantly increased (396 ± 50 versus 250 ± 24 nmol/liter, p < 0.05). There were no significant differences in levels of diastolic free Ca\(^{2+}\) concentration (181 ± 11 versus 171 ± 14 nmol/liter, p = not significant). The time for 70% Ca\(^{2+}\) uptake was also accelerated in TG myocytes compared with WT myocytes (78 ± 6 versus 141 ± 8 ms, p < 0.05). Thus, both peak Ca\(^{2+}\) levels and Ca\(^{2+}\) uptake are increased in myocytes isolated from TG. Myocytes isolated from Tg-KI-Akt did not show any significant changes in peak Ca\(^{2+}\) levels and Ca\(^{2+}\) uptake. These results indicate that changes in contractile function and Ca\(^{2+}\) transients are dependent upon Akt activity.

L-type Ca\(^{2+}\) Channel Currents—Because Ca\(^{2+}\) influx through L-type Ca\(^{2+}\) channels plays an essential role in excitation-contraction coupling, we examined L-type Ca\(^{2+}\) currents (\(i_{\text{Ca}}\)) in LV myocytes isolated from TG and WT (Fig. 2). LV myocyte size estimated by cell capacitance was significantly larger in TG myocytes compared with WT myocytes (Fig. 2B), consistent with the heart weight/body weight ratio data.

The traces in Fig. 2 show typical \(i_{\text{Ca}}\) recorded from TG and WT myocytes. In both groups, \(i_{\text{Ca}}\) amplitude, normalized by the cell capacitance (pA/pF) was significantly larger (p < 0.01) in TG myocytes (9.0 ± 0.3 pA/pF) compared with WT myocytes (7.2 ± 0.3 pA/pF). The inactivation kinetics of \(i_{\text{Ca}}\) and relaxed in TG myocytes compared with WT myocytes (Fig. 2B). In mouse ventricular myocytes, \(i_{\text{Ca}}\) inactivation is controlled by Ca\(^{2+}\) in the sarcoplasmic subspace (19, 23, 24). To examine whether the difference in \(i_{\text{Ca}}\) inactivation rate between WT and TG myocytes was due to the contribution of Ca\(^{2+}\) release from the SR, the SR Ca\(^{2+}\) content was depleted by ryanodine (10 μM). Following the application of ryanodine, the rate of \(i_{\text{Ca}}\) inactivation as measured by \(t_{\text{1/2}}\) was significantly increased in both WT and TG myocytes, and no significant difference in the inactivation rate was detected between two groups (31.4 ± 2.3 ms, n = 6 versus 29.5 ± 2.1 ms, n = 6). The results indicate that the faster \(i_{\text{Ca}}\) inactivation observed in TG myocytes might be related to enhanced SR Ca\(^{2+}\) release (See below).

SERCA2a, PLB, Calsequestrin, α\(_{\text{I}}\), NCX, and Ryanodine Receptor Protein—Because both enhanced amplitude and relaxation of the Ca\(^{2+}\) transient may be caused by changes in SR Ca\(^{2+}\)-handling proteins, the expression of these proteins was determined by immunoblot analyses. The protein expression of Akt in myocardium was increased ~25-fold in TG compared with WT mice. SERCA2a protein levels were increased significantly (p < 0.05) by 6.6-fold in TG compared with WT mice. In contrast, protein levels of PLB, calsequestrin, α\(_{\text{I}}\), and NCX were not significantly different between TG and WT mice (Fig. 3, A and B). The affinity and number of ryanodine receptors were similar between WT and TG (Fig. 3C). Thus, the increase in SERCA2a protein in TG occurs post-transcriptionally.

Overexpression of Akt Increases Expression of SERCA2a in Adult Ventricular Cardiac Myocytes—To determine whether transient overexpression of Akt is sufficient to increase SERCA2a protein expression in cardiac myocytes, we conducted adenovirus-mediated overexpression of Akt in primary cultured adult rat ventricular cardiac myocytes. Forty-eight hours after transfection, overexpression of Akt was confirmed by immunoblot analyses. Immunoblotting of the same filters with anti-SERCA2a antibody indicated that there was no significant difference in SERCA2a mRNA levels between TG and WT (Fig. 3C). Thus, the increase in SERCA2a protein in TG occurs post-transcriptionally.

Fig. 1. A, length of adult ventricular cardiac myocytes isolated from WT and TG. B, Ca\(^{2+}\) transients in adult ventricular cardiac myocytes isolated from WT and TG.
Up-regulation of SERCA2a Increases Contractile and Relaxation Function in Isolated Cardiac Myocytes from TG

We examined the effect of thapsigargin, a specific inhibitor of SERCA2a, on contraction of isolated myocytes. The dose of thapsigargin inhibiting % contraction by half (IC50) was 2.6-fold higher in myocytes isolated from TG than in those from WT (Fig. 5). Enhanced relaxation function in TG myocytes was also abolished after thapsigargin (10^{-6} M). These results are consistent with the notion that enhanced SERCA2a plays an important role in mediating increased cardiac myocyte contraction and relaxation in TG.

DISCUSSION

Akt plays a central role in glucose metabolism, cell growth, angiogenesis, transcription, apoptosis, and protein synthesis (25). We found that overexpression of constitutively active Akt

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**Table II**

| Parameters | WT | TG | Tg-KI-Akt |
|------------|----|----|-----------|
| % Contraction | 3.5 ± 0.2 | 6.1 ± 0.2* | 3.6 ± 0.2 |
| -dL/dt (μm/s) | -147 ± 20 | -284 ± 15* | -158 ± 3 |
| relaxation | 98 ± 18 | 214 ± 15* | 116 ± 5 |
| tR 70% (ms) | 67 ± 5 | 48 ± 2* | 69 ± 2 |
| No. of cells studied | 81 | 105 | 62 |
| [Ca^{2+}]_i, Diastolic (nmol/liter) | 181 ± 11 | 171 ± 14* | 166 ± 17 |
| Systolic (nmol/liter) | 421 ± 33 | 567 ± 63* | 421 ± 50 |
| Amplitude (nmol/liter) | 250 ± 24 | 396 ± 50* | 255 ± 36 |
| tR 70% (ms) | 141 ± 8 | 78 ± 6* | 131 ± 13 |
| No. of cells studied | 55 | 63 | 51 |

* p < 0.05 versus WT. Values are mean ± S.E.

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**Fig. 2. A**, whole-cell \(I_{\text{Ca}}\) recorded in WT and TG myocytes. Traces show currents elicited from a holding potential of -50 mV to the indicated test potentials. TG myocytes had larger \(I_{\text{Ca}}\) amplitudes and faster inactivation kinetics. **B**, average cell capacitance, \(I_{\text{Ca}}\) density, and half-maximal decay \(t_{1/2}\) of \(I_{\text{Ca}}\) obtained from WT and TG myocytes. Data points are mean ± S.E. The numbers correspond to total number of cells measured. An asterisk indicates that the mean values are significantly different (\(p < 0.01\)) from respective WT controls.
Akt Induces $Ca^{2+}$-mediated Enhanced Myocyte Function

**Fig. 3.** A and B, Western blotting of Akt, total PLB, calsequestrin (CQN), SERCA2a, $\alpha_{1c}$, and NCX from myocardial extracts from WT ($n = 4$) and TG ($n = 4$). In A, representative gel pictures are shown. In B, the intensity of each band is expressed in arbitrary units. An asterisk indicates that the mean values are significantly different ($p < 0.01$) from respective WT controls. C, a representative result of the reverse transcription-PCR analyses of SERCA2a and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) is shown.

**Fig. 4.** Western blotting of SERCA2a (top) and Akt (bottom) from LacZ (lanes 1 and 2) and Akt-transfected (lanes 3 and 4) adult myocyte cultures, demonstrating that the overexpression of Akt up-regulates SERCA2a protein. Note that SERCA2a was detected as a doublet in control virus-transfected myocytes, whereas only a slower migrating form was detected in Akt-transfected myocytes.

in the mouse heart increased both LV ejection fraction, *in vivo*, and isolated myocyte contraction *in vitro*. A recent study using the same TG model demonstrated increased LV dP/dt with a nonsignificant increase in LV ejection fraction (11). The failure to observe significantly increased LV ejection fraction in the prior study (11) may be due to heart rate, which was significantly lower in TG than WT, whereas the heart rates were similar in the two groups in the present study. Interestingly, the improved LV function occurred in the presence of significant LV hypertrophy, which is thought to reduce LV function. The goals of the present study were: 1) to investigate whether constitutively active Akt has direct effects upon contractility and $Ca^{2+}$ handling in isolated cardiac myocytes; and if so, 2) to identify the downstream mechanism of the enhanced cardiac myocyte function by Akt activation.

We have demonstrated that the enhanced cardiac function in TG *in vivo* is associated with increased intrinsic contractile and relaxation function in isolated cardiomyocytes *in vitro*. Moreover, the increased contraction and relaxation in myocytes isolated from TG paralleled the increased peak systolic amplitude and more rapid diastolic decay in intracellular $Ca^{2+}$ transients. The studies in isolated myocytes also demonstrated increased $Ca^{2+}$ channel currents. These functional changes in isolated myocytes were not observed in Tg-KI-Akt, suggesting that they are dependent upon the kinase activity of Akt. This indicates that activation of Akt directly affects contraction and relaxation through changes in $Ca^{2+}$ handling in individual cardiac myocytes.

It should be noted that the enhanced cardiac contractility was not observed in TG mice with cardiac-specific overexpression of constitutively active PI3K, an upstream regulator of Akt (26), or in other forms of constitutively active Akt (T308D, S473D-Akt, and myr-Akt) (10, 27) distinct from the one used in this study (E40K-Akt), despite the fact that all mice exhibited cardiac hypertrophy. Furthermore, phosphatidylinositol 3-kinase-γ activated by inhibition of PTEN (phosphatase and tensin homologue deleted on chromosome 10) negatively regulates cardiac contractility (28). Although myr-Akt is constitutively localized at the plasma membrane, both wild type Akt and E40K-Akt undergo growth factor-dependent membrane translocation (29). These results suggest that cardiac contractility may be tightly regulated by activation of a specific component of the phosphatidylinositol 3-kinase pathway and/or by subcellular localization or the substrate specificity of the Akt mutants used. Thus, it is possible that E40K and wild type Akt may share downstream targets for the enhanced cardiac function.

To identify the downstream mechanisms of Akt responsible for enhanced myocyte contraction and relaxation, we examined mechanisms known to control intracellular $Ca^{2+}$ transients. We found that the amplitude of $I_{Ca}$ was enhanced in Akt myocytes by $\sim 20\%$ compared with that in WT myocytes, which may be at least in part responsible for the enhanced cellular $Ca^{2+}$ transients. Akt mediates insulin-like growth factor-induced potentiation of $I_{Ca}$ in neuronal cells (30). Therefore, to determine whether the increased $I_{Ca}$ was due to altered $Ca^{2+}$ channel expression, we measured the expression level of the $\alpha$-subunit of the cardiac L-type $Ca^{2+}$ channel ($\alpha_{1c}$) and found no significant differences in $\alpha_{1c}$ protein expression between WT and TG. These results indicate that the enhanced $I_{Ca}$ amplitude observed in Akt myocytes may be related to the altered channel regulation, *i.e.* an increase in open probability or con-
Ca2+ entry through L-type Ca2+ channels is a critical first step in the Ca2+-handling cascade. The cytosolic Ca2+ transients and contraction elicited by membrane depolarization are strongly influenced by the amount of Ca2+ influx through the channel. Thus, an increase in Ca2+ entry through the L-type Ca2+ channels is likely to contribute to the increase in cellular Ca2+ transients observed in TG myocytes (31). It is also possible that Akt could alter the Ca2+ dependence of the open probability of the ryanodine receptor. Our results, however, suggest that neither the affinity nor the total number of ryanodine receptors was affected by activation of Akt.

In normal cardiac myocytes isolated from rats or mice, ~90% of cytosolic Ca2+ is removed through SERCA (32). Overexpression of SERCA2a or the enhanced SERCA activity by phosphorylation of PLB accelerates Ca2+ transients and cardiac relaxation (33, 34). In the present study, SERCA2a protein levels were up-regulated in TG mice. Interestingly, we found that higher doses of thapsigargin are required to reduce % contraction in cardiac myocytes isolated from TG and the enhanced relaxation function in TG myocytes was abolished in the presence of thapsigargin. These results strongly suggest that increased levels of SERCA2a lead to enhanced Ca2+ transients and contraction as well as accelerated relaxation in cardiac myocytes. Our results indicate that up-regulation of SERCA2a expression by Akt is mediated by post-transcriptional mechanisms. The amino acid sequence of mouse SERCA2a contains potential phosphorylation sites by Akt. Our experiments using cultured adult ventricular cardiac myocytes indicated that adenovirus-mediated transient overexpression of Akt increased expression of SERCA2a. Furthermore, on the SDS-PAGE, only a slower migrating form of SERCA2a appeared after Akt overexpression. This slower migrating form disappeared after phosphatase treatment, and thereafter only the faster migrating form was observed (data not shown), consistent with the concept that SERCA2a is phosphorylated, when Akt is overexpressed. Whether SERCA2a is a physiological substrate of Akt, and if so, whether activation of Akt enhances translation or stability of SERCA2a, remains to be elucidated.

We and others have shown that activation of Akt causes cardiac myocyte hypertrophy both in vitro and in vivo (7, 10, 11, 27). Because ventricular hypertrophy is usually accompanied by decreases in SERCA2a, which lead to impairment of Ca2+ handling, up-regulation of SERCA2a and acceleration of Ca2+ transients despite the induction of hypertrophy in this transgenic model is unique, particularly in view of the well known effects of hypertrophy in interfering with Ca2+ handling and myocyte contraction (35, 36). Because Akt prevents cardiac myocyte death in response to pathologic stimuli (16), stimulation of Akt might be an ideal method of enhancing the contractility of the heart and improving Ca2+ handling in response to pressure overload.

In summary, transgenic overexpression of protein kinase Akt enhances myocyte contractility and relaxation through accelerated intracellular Ca2+ transients. The underlying cellular mechanism(s) include potentiation of L-type Ca2+ channel function and up-regulation of SERCA2a.

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