Transgenic Overexpression of Heart-specific Adenine Nucleotide Translocase 1 Positively Affects Contractile Function in Cardiomyocytes

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Key Words

Adenine nucleotide translocase • Contraction-relaxation cycle • Heart • Cardiomyocytes • Myofibrillar proteins • Transgenic rat

Abstract

Background/Aims: The adenine nucleotide translocase (ANT) exchanges ATP and ADP over the inner mitochondrial membrane, supplying the cells with energy. Interestingly, myocardial ANT1 overexpression preserves cardiac structure and function under pathophysiological conditions. To ascertain whether the contractile system is directly affected by increased ANT1 expression, we analyzed cell morphology, contraction and relaxation parameters of ANT1 transgenic (ANT1-TG) cardiomyocytes, myofibrillar protein expression, and Ca²⁺ handling in ANT1-TG rat hearts. **Results:** ANT1-TG cardiomyocytes displayed an elevation in cell volume (52.6±12.0%; p<0.0001) in comparison to wildtype (WT) cells. Concurrently, contractile function in ANT1-TG cells was significantly increased, measured by a decline

in time to peak contraction (TTP) and RT50, the time from peak contraction to 50% relaxation, during stimulation with 0.5, 1, and 2 Hz. Quantification of myofibrillar proteins exhibited a marked increase in total cardiac myosin heavy chain (51.8±12.8%) (p<0.03), beta myosin heavy chain (22.9±5.0%; p<0.03), actin (23.8±8.8%; p<0.05), and troponin I (51.5±13.7%; p<0.01). Regarding intracellular Ca²⁺ handling, ANT1-TGs revealed a significant elevation in sarcoplasmic reticulum (SR) Ca²⁺ ATPase (SERCA2a) protein level (22.2±4.7%; p<0.01) associated with increased Ca²⁺ uptake into the SR (34%; p<0.01). Moreover, the plasmalemmal Ca²⁺ ATPase (PMCA) indicated advanced protein expression (23.8±4.8%; p<0.01), whereas the protein amount of the Na⁺/Ca²⁺ exchanger was not altered in ANT1 overexpressing hearts. **Conclusion:** These data reveal a close association of elevated mitochondrial ATP/ADP transportation via ANT1 with increased contractile function. Furthermore, the ANT1-TGs exhibit an elevation in SR Ca²⁺ transport that contributes to increased cardiac work, which may protect the heart under pathophysiological conditions.

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Introduction

The adenine nucleotide translocase (ANT) facilitates the exchange of mitochondrial ATP and cytosolic ADP across the inner mitochondrial membrane and thus couples both energy-producing and energy-consuming processes [1]. The dimeric ANT complex of rodents is nuclear-encoded by three distinct genes (ANT1, ANT2, and ANT4) that are co-expressed in tissue-specific patterns [2, 3]. ANT1 is the predominant isoform in heart mitochondria.

Knockout of ANT1 has been shown to result in mitochondrial energetic insufficiency that causes severe cardiac hypertrophy [4]. In general, hypertrophic hearts demonstrate a dramatic destruction of myocyte organization and altered sarcomere structure especially shown by a downregulation of contractile material like alpha myosin heavy chain [5]. These defects lead to a loss in contractile function accompanied by abnormal cellular Ca^{2+} handling caused by impaired Ca^{2+} transport processes in the sarcolemma and the sarcoplasmic reticulum (SR). In particular, the return of cytosolic Ca^{2+} into the SR, the major contributing process for cardiac relaxation, which is catalyzed by the SR Ca^{2+} ATPase SERCA2a, is restricted [5].

Conversely, previous investigations have demonstrated that heart-specific, transgenic overexpression of ANT1 increased the efficiency of mitochondrial ATP/ADP transportation and energetic coupling of the oxidative phosphorylation. Moreover, ANT1 overexpression prevented tissue destruction and cardiac dysfunction in hypertension-induced hypertrophy and diabetic cardiomyopathy [6, 7]. Thus, ANT1 overexpression induces an intracellular cardioprotective program that influences various intracellular processes [8].

To investigate whether ANT1 overexpression directly affects the myocardial contractile system, cell morphology and contractile parameters of single cardiomyocytes as well as the expression of myofibrillar proteins were determined. In addition, we analyzed the abundance of reticular and sarcolemmal Ca^{2+} transporting proteins and the SR Ca^{2+} transport function in ANT1-TG rat hearts.

Materials and Methods

Transgenic animals

The transgenic rat strain with cardiac overexpression of rat ANT1 (TGRMHCrANT1 [ANT]) was generated using the

rat α -myosin heavy chain promoter as first described in Walther et al. [6]. Hearts of male WT and ANT1-TG rats were quickly excised and immersed in an ice-cold solution containing 130 mmol/L NaCl, and 30 mmol/L KCl. Left ventricular (LV) tissue samples were shock-frozen in liquid nitrogen and stored at -80°C until analysis. The animal studies were performed according to national guidelines and were approved by the institutional animal care committee.

Cell cultures and treatment

Ventricular cardiomyocytes were isolated from 250 to 300 g male Sprague Dawley rats, suspended in basal culture medium and plated on culture dishes, which were preincubated overnight with 4% fetal calf serum in medium 199, as previously described [9]. The basal culture medium (CCT) was modified medium 199 including Earle's salts, 2 mmol/L L-carnitine, 5 mmol/L taurine, 100 IU/ml penicillin, 100 $\mu\text{g}/\text{ml}$ streptomycin and 10 $\mu\text{mol}/\text{L}$ cytosine- β -D-arabinofuranoside (pH 7.4). Two hours after plating, the dishes were washed twice with CCT medium. This resulted in cultures of about 90% quiescent rod-shaped cells on average.

Cell morphology

Myocyte size was determined on micrographs digitalized by a charge-coupled device camera, as described elsewhere [10]. Five micrographs per sample were randomly taken, and all rod-shaped myocytes in these fields were measured. This resulted in a total of 60–100 cells measured for one condition of one preparation. Diastolic cell length and width/diameter of myocytes were determined at the widest point of each myocyte using the software program "Analysis from SIS". Cross-sectional area and cell volume of cardiomyocytes were calculated by the formulas $(\text{radius})^2 \times \pi$ and $(\text{radius})^2 \times \pi \times \text{lengths}$, respectively, assuming a cylindrical cell shape.

Determination of cell contraction

Cells were allowed to contract at room temperature and analyzed using a cell-edge detection system. Cells were stimulated via two AgCl electrodes with biphasic electrical stimuli composed of two equal but opposite rectangular 50 V stimuli of 0.5 ms duration. Each cell was stimulated at 0.5 Hz, 1 Hz and 2 Hz for 1 min. Every 15 seconds the next five contractions were averaged. The mean of these four measurements at a given frequency was used to define the contractility of a given cell. Cell length was measured at a rate of 500 Hz via a line camera. As parameters of contractile function, fractional cell shortening (dL/L), time to peak contraction (TTP) and time from peak contraction to 50% relaxation (RT50) were determined.

Preparation of cardiac homogenates

Tissue homogenization was performed by five strokes each at 21 000 revolutions/min for 10 seconds in 30 volumes of ice-cold extraction buffer using a Brinkmann Polytron PT 3000 (Kinematica, Littau/Lucerne, Switzerland) equipped with a PT-DA3007/2 piston. The extraction buffer contained 50 mmol/L KH_2PO_4 , 15 mmol/L NaF, 1 mmol/L EDTA, 250 mmol/L sucrose, 0.3 mmol/L phenylmethylsulfonyl fluoride (PMSF), 0.5 mmol/L DL-dithiothreitol (DTT), 1/100 volume protease inhibitor cock-

Fig. 1. Morphology of WT and ANT1-TG cardiomyocytes. (A) Generic micrographs of single cardiomyocytes (magnification $\times 20$). (B) Diastolic cell length and (C) cardiomyocyte width were determined at the widest point of each myocyte and depicted in % of control. (D) Cross-sectional area (CSA) and (E) cell volume were consistently calculated from cell radius and cell length. Data are given as mean $\pm$ SEM for 120 WT cells and 96 ANT1-TG cells of $n=2$ independent preparations. * $p<0.05$ vs. WT controls, ** $p<0.01$, *** $p<0.0001$

tail as well as 10 mmol/L histidin (pH 7.4). The homogenate was filtrated and the protein amount was determined according to Lowry's method, using ovalbumin as standard after incubating samples in 1 mol/L NaOH for 30 min. Samples were shock-frozen and stored at -80°C until use. Cardiac homogenates were utilized for western blot analysis and calcium transport measurements.

Western blot analysis

2 μg –30 μg of protein were separated by electrophoresis using 4–12% gradient polyacrylamide gels (Invitrogen, Darmstadt, Germany) and blotted onto a polyvinylidene difluoride membrane for 90 min. Western blots were performed using a standard protocol with specific primary antibodies (see below), horse-radish peroxidase-conjugated goat anti-rabbit secondary antibody and goat anti-mouse secondary antibody (Dako, Glostrup, Denmark), respectively. Protein specific signals were normalized against loaded protein visualized by Coomassie Blue. Primary antibodies used were: anti-heavy chain cardiac myosin [3–48], anti-calcium pump pan PMCA ATPase [5F10], anti-calcium channel L-type DHPR alpha 2 subunit antibody [20A] (Abcam, Cambridge, USA), anti-myosin (skeletal, slow), clon NOQ7.C.AD (Sigma-Aldrich, Hamburg, Germany), anti-actin (C-2), anti-troponin I (FL-210), anti-calsequestrin 2 (H-60) (Santa Cruz Biotechnology, Inc, Heidelberg, Germany), anti- $\text{Na}^+/\text{Ca}^{2+}$ exchanger (Swant, Bellinzona, Switzerland), anti-ryanodine receptor (C3-33) (Calbiochem, Darmstadt, Germany), anti-SERCA2a (antiserum, gift of Dr. W. Dillmann, San Diego), and anti-phospholamban, clon A1 (Millipore, Hamburg, Germany).

Calcium transport measurements

Initial rates of SR oxalate-supported Ca^{2+} uptake were estimated in LV homogenates at 37°C using a standard procedure of Solaro & Briggs [11] that had been modified and described by us earlier [12]. Under these specified conditions, Ca^{2+} uptake has been defined to be restricted to SR vesicles in the homogenates [11–13]. Briefly, the preheated reaction medium contained 40 mmol/L imidazole (pH 7.0), 100 mmol/L KCl, 5 mmol/L MgCl_2 , 5 mmol/L Tris-ATP, 6 mmol/L phosphocreatine, 10 mmol/L K-oxalate, 0.2 mmol/L EGTA, 10 mmol/L NaN_3 ,

2 $\mu\text{mol/L}$ synthetic protein kinase A inhibitor peptide [PKI(6–22)amide] and 0.35 $\mu\text{mol/L}$ or 3.68 $\mu\text{mol/L}$ free $^{45}\text{CaCl}_2$ (1.9×10^{11} Bq/mol), respectively. Measurements were started by adding 30 μg homogenate protein to 0.15 ml of the reaction mixture. A sample of 0.15 ml taken after 2 min was filtered through 0.45 μm Millipore filters using a vacuum pump. Filters were washed twice with 3 ml ice-cold solution containing 100 mmol/L KCl, 2 mmol/L EGTA, and 40 mmol/L imidazole (pH 7.0) and dried for 30 min at 60°C . All measurements were performed in duplicate. The $^{45}\text{CaCl}_2$ associated with the dried filters was determined by liquid scintillation counting. In addition to these conditions, Ca^{2+} uptake measurements at 3.68 $\mu\text{mol/L}$ free $^{45}\text{CaCl}_2$ were made in the presence of 20 $\mu\text{mol/L}$ Ruthenium Red. The latter conditions were used to reduce the Ca^{2+} leakiness of ryanodine receptors (RyRs) within the membrane of SR vesicles. In control experiments, thapsigargin and the ionophore A23187 at 10 $\mu\text{mol/L}$ prevented Ca^{2+} uptake completely. The rates of Ca^{2+} uptake were expressed as nanomoles of Ca^{2+} per milligram of membrane protein per minute.

Statistical analysis

Statistical analysis was performed by Student's t test or the Mann-Whitney U test. Values are shown as mean $\pm$ SEM of n independent experiments. Differences were considered significant at values of $p<0.05$.

Results

Morphology of ANT1-TG cardiomyocytes

Cardiac performance depends on structural properties of single cells. Microscopic analysis of adult cardiomyocytes isolated from ANT1-TG hearts displayed an increase in diastolic cell length (Fig. 1B; $6.3 \pm 2.0\%$, $p<0.05$) associated with a significant enlargement in cell width (Fig. 1C; $19.3 \pm 4.8\%$, $p<0.01$) in comparison to WT. The increasing cell width results in a larger cross-sectional area (CSA) (Fig. 1D; $43.8 \pm 14.1\%$, $p<0.01$) and

consequently leads to an elevation in cell volume (Fig. 1E; $52.6 \pm 12.0\%$, $p < 0.0001$).

ANT1 overexpression increases contractile function in rat cardiomyocytes

To determine the contractile function in ANT1-TG cells, contractile parameters were measured during stimulation with increasing electrical frequencies. We focused on parameters such as fractional cell shortening (dL/L), time to peak contraction (TTP) and time from peak contraction to 50% relaxation (RT50). In general, rat ventricular cardiomyocytes display a negative cell shortening/frequency relationship as shown in Fig. 2A. The fractional cell shortening amplitude was not different between ANT1-TG and WT cells. In contrast, the load independent time parameter TTP and RT50 were significantly reduced in ANT1-TG cardiomyocytes at frequencies of 0.5 Hz, 1 Hz and 2 Hz of stimulation (Fig. 2B, 2C). These data demonstrate enhanced contractile function of ANT1-TG cardiomyocytes in comparison to WT cells.

ANT1-TG rat hearts reveal an increase in myofibrillar proteins

We assumed that the increase in contractile function is related to additional contractile material in ANT1-TG hearts. Assessment of total LV myosin heavy chain (MHC) protein demonstrated a significant elevation of $51.8 \pm 12.8\%$ ($p < 0.03$) in ANT1-TG rats compared to WT animals (Fig. 3A). This finding was associated with a significant increase in beta myosin heavy chain (β MHC) (Fig. 3B; $22.9 \pm 5.0\%$; $p < 0.03$). Further examination revealed a remarkable elevation in actin (Fig. 3C; $23.8 \pm 8.8\%$; $p < 0.05$) and troponin I (TnI) expression in ANT1-TG rats (Fig. 3D; $51.5 \pm 13.7\%$; $p < 0.01$).

ANT1 overexpression leads to an upregulation of Ca^{2+} -transporting ATPases

To investigate whether Ca^{2+} handling is adapted to the expanded contractile system and contributes to enhanced contractile function, we determined protein amounts of Ca^{2+} -transporting components. First, we focused on the dihydropyridine receptor (DHPR) and the ryanodine receptor 2 (RyR2), two Ca^{2+} release channels found in the cell membrane of the cardiac muscle and SR membrane, respectively. Protein amounts of DHPR and RyR2 remained unchanged in ANT1-TG animals (data not shown). The expression of calsequestrin, a Ca^{2+} -binding protein within the SR was also not altered (data not shown).

Fig. 2. Contractile parameters of isolated WT (square) and ANT1-TG cardiomyocytes (rhombus). Myocytes were stimulated for 1 min with increasing electrical frequencies (0.5 Hz, 1 Hz and 2 Hz) and contractile parameters were measured using a cell-edge detection system. Data on (A) fractional cell shortening (dL/L), (B) time to peak contraction (TTP) and (C) RT50, characterized as time from peak contraction to 50% relaxation were collected. Data are given as mean $\pm$ SEM for 18 cells of $n=6$ independent preparations. * $p < 0.05$ vs. WT controls, *** $p < 0.0001$

Cardiac relaxation is initiated by Ca^{2+} reuptake via SERCA2a, the plasma membrane Ca^{2+} ATPase (PMCA) and the $\text{Na}^+/\text{Ca}^{2+}$ exchanger (NCX). The protein amount of PMCA and SERCA2a revealed a considerable increase (Fig. 4A; $23.8 \pm 4.8\%$; $p < 0.01$ and Fig. 4B; $22.2 \pm 4.7\%$; $p < 0.01$) in ANT1-TG hearts, while the protein level of the NCX did not differ between transgenic animals and WT controls (Fig. 4A). The protein amount of phospholamban (PLB), an inhibitory protein of SERCA2a, was not altered in ANT1-TG rat hearts

(Fig. 4B), thus the calculated PLB/SERCA2a ratio was markedly reduced in ANT1-TGs (Fig. 4C; $29.0 \pm 5.6\%$; $p<0.06$). A reduction in the PLB/SERCA2a ratio can directly increase SR Ca^{2+} uptake function and muscle contractility.

SERCA2a overexpression increases SR Ca^{2+} uptake in vitro

To further examine whether enhanced expression of SERCA2a leads to an elevation in SR Ca^{2+} transport activity, we determined the oxalate-supported SR Ca^{2+} uptake in LV homogenates (Fig. 5). In ANT1-TG animals, the rate of Ca^{2+} uptake was 34% ($p<0.01$) higher than in WT controls. This was observed at both submicromolar ($0.35 \mu\text{mol/L}$) and saturating ($3.68 \mu\text{mol/L}$) free Ca^{2+} concentrations. Ca^{2+} transport activity at the submicromolar free Ca^{2+} concentration reaches 50% of the respective maximum Ca^{2+} uptake values measured at a saturating Ca^{2+} concentration in both groups.

To clarify whether membrane leakiness of Ca^{2+} over the RyR contributed to the observed differences in Ca^{2+} uptake rate, measurements of SR Ca^{2+} uptake were done in the presence of the RyR inhibitor ruthenium red (RR). Inhibition of RyRs by $20 \mu\text{mol/L}$ ruthenium red caused a similar increase in net Ca^{2+} uptake by 65% in both groups and thus did not alter the above-mentioned intergroup difference in SR Ca^{2+} uptake.

Discussion

Based on ANT's physiological significance, up-regulation and down-regulation of ANT expression have profound effects on cellular processes [4, 6]. This study has demonstrated that an overexpression of ANT1 in

transgenic rat hearts increases the cell size, which is linked to an acceleration in contraction and myofibrillar protein abundance. In addition, ANT1-TGs exhibit an increased expression of the sarcolemmal PMCA and the reticular Ca^{2+} ATPase SERCA2a. The latter contributes to an enhanced SR Ca^{2+} uptake function and accelerated contraction and relaxation.

Contractile measurements on cardiomyocytes at different frequencies of stimulation have demonstrated a significant elevation in contractile performance of ANT1-TGs determined by the load independent time parameters, TTP and RT50. In addition, microscopic analysis of individual ANT1-TG cardiomyocytes revealed a significant increase in cell lengths and cell width resulting in an elevation of cell size and thus indicating cellular hypertrophy. This cellular hypertrophy is linked to increased total wet heart weight, which is consistent to a significant elevation in left ventricular end-systolic and end-diastolic volume, indicating an increase in left ventricular chamber size. The increase in heart weight was accompanied by a higher body weight, thus the calculated ratio of heart to body weight was slightly but not significantly enlarged [6].

We assume that changes in cardiac cell structure may contribute to elevated contractile function due to increased abundance of myofibrillar proteins. Indeed, assessment of total MHC, the most abundant protein in cardiac muscle, has demonstrated a significant elevation in ANT1-TG rats compared to WT animals. Homodimers of α MHC and β MHC are the predominant isoforms of myosin in cardiac tissue. Rat hearts exhibit approx. 80% α MHC and 20% β MHC [14]. In general, the homodimer of β MHC is regarded as slow myosin, whereas the α MHC homodimer is the fast myosin isoform [15]. β MHC protein was only slightly increased in ANT1-TG myocar-

Fig. 4. Protein amounts of Ca^{2+} transporting components in response to ANT1 over-expression. Protein extracts were prepared from cardiac tissue isolated from WT and ANT1-TG rats and amounts of Ca^{2+} transporting proteins determined by western blot analysis. The left panel shows representative western blots and the right panel displays the quantification of proteins in % of control. Figure (A) represents the $\text{Na}^+/\text{Ca}^{2+}$ exchanger (NCX) and the plasma membrane Ca^{2+} ATPase (PMCA). Figure (B) shows the SR Ca^{2+} ATPase (SERCA2a) and the low molecular weight form of phospholamban (PLB). Figure (C) indicates the calculation of PLB to SERCA2a ratio. Protein specific signals were normalized against loaded protein visualized by Coomassie Blue. Data are given as mean $\pm$ SEM for $n=8-12$ each. ** $p<0.01$ vs. WT controls, # $p<0.06$

dium, thus the main increase in total MHC occurred by α MHC protein expression. This tremendous increase in MHC protein was in line with a significant elevation in other thin filament proteins like actin and troponin I in ANT1-TG rats. Besides, former studies with electron microscopic analysis of longitudinal sections displayed no alterations in sarcomere structure and WT and ANT1-TGs exhibit a regular array of myofibrils with distinct I-bands and A-bands in addition to well-defined Z-bands and M-lines [6]. Thus, the increased myofibrillar proteins do not disturb sarcomere organization. ANT1-TG cells seem to assemble newly formed myofibrils with an increased content of α MHC that could contribute to the observed accelerated contraction.

An acceleration in contraction is often accompanied by increased cellular Ca^{2+} handling. Basically, SERCA2a, PMCA, and the sarcolemmal $\text{Na}^+/\text{Ca}^{2+}$ exchanger remove cytosolic Ca^{2+} after contraction, leading to cardiac muscle relaxation. Interestingly, we found an increased protein abundance of the Ca^{2+} ATPases SERCA2a and PMCA in ANT1-TG rats, while protein amounts of the $\text{Na}^+/\text{Ca}^{2+}$ exchanger did not differ between transgenic animals and WT controls. While PMCA contributes little to diastolic Ca^{2+} decline [16, 17], the rate of Ca^{2+} removal from the cytosol is mainly determined by SERCA2a-catalyzed SR Ca^{2+} uptake activity [17-19]. In LV cardiac homogenates, the SERCA2a-catalyzed SR Ca^{2+} uptake rate was found to be 34% higher in ANT1-TG rat hearts than in WT controls. These data suggest that the expression of additional SERCA2a molecules causes the increase in SR Ca^{2+} pumping activity. Because relaxation is largely determined by SR Ca^{2+} uptake, the observed acceleration in RT50 of isolated

Fig. 5. Differences in sarcoplasmic reticular Ca^{2+} uptake in left ventricular homogenates prepared from WT and ANT1-TG rat hearts. Oxalate-supported Ca^{2+} uptake was measured at submicromolar ($0.35 \mu\text{mol/L}$) and saturating ($3.68 \mu\text{mol/L}$) free $^{45}\text{CaCl}_2$ concentrations. To exclude membrane leakiness of the ryanodine receptors for Ca^{2+} during the experiment, $20 \mu\text{mol/L}$ ruthenium red (RR) was added to the reaction mixture. The rate of Ca^{2+} uptake is expressed as nanomoles of Ca^{2+} per milligram protein per minute. Data are given as mean $\pm$ SEM for $n=7-8$ each. ** $p<0.01$ vs. WT controls

cardiomyocytes appeared be due to the observed increase in SERCA2a activity. These results are in agreement with a great number of studies indicating that transgenic overexpression of SERCA2a in the heart results in faster rates of Ca^{2+} decline and increased contractility [13, 20-23]. For example, an increased SERCA2a protein level in the atria of rodents is accompanied by higher pump levels, which may at least in part account for the shorter duration of contraction in atrial vs. ventricular tissue [24]. In addition, del Monte et al. [20] showed that

overexpression of SERCA2a in human ventricular myocytes from patients with end-stage heart failure can increase SERCA2a pump activity and enhance contractile function.

In the heart, SERCA2a pump activity is mainly regulated by unphosphorylated phospholamban (PLB) that keeps the Ca^{2+} affinity of SERCA2a low and results in decreased SR Ca^{2+} uptake, slowed relaxation and decreased SR Ca^{2+} load. PLB phosphorylation in response to β -adrenergic responses relieves this inhibition [25, 26]. A decrease in the unphosphorylated PLB to SERCA2a ratio of about 34% as observed in ANT1-TG hearts is in line with the elevation in SERCA2a activity and may contribute to the faster relaxation.

In general, SERCA2a is the second largest consumer of ATP after the myosin ATPase, and therefore SERCA2a activity could be affected by changes in energetics and energy supply [27]. In fact, the SR Ca^{2+} ATPase seems to be the most sensitive ATPase in response to the reduction in free energy released from ATP hydrolysis ($\Delta G_{\sim p}$), and other studies suggest that a decrease in $\Delta G_{\sim p}$ may directly translate into defects in contractile function [28, 29]. By contrast, the ANT1 overexpression in transgenic rat hearts generates an accelerated ATP/ADP transportation in mitochondria and enhances oxidative phosphorylation by increasing complex activities II-IV [6]. Thus, the accelerated energy transfer may be able to support more SERCA2a molecules with ATP and thereby contribute to increased contractile function.

In myocytes, with a given specific preload and equipment of contractile proteins, the amplitude of contraction is determined by systolic Ca^{2+} transients induced principally through L-type Ca^{2+} channels (DHPR) activating SR Ca^{2+} release through ryanodine receptors (RyRs) [30]. Both, the DHPR and RyR revealed no change in protein amount between WT and ANT1-TGs. Because the magnitude of SR Ca^{2+} release is principally a function of SR Ca^{2+} load [31-33], we suggest that the elevated SERCA2a activity, observed in ANT1-TGs presumably increase the free SR Ca^{2+} concentration because calsequestrin, an intra-SR Ca^{2+} buffer, remained unchanged. Therefore, the free SR Ca^{2+} content may el-

evate the fractional SR Ca^{2+} release and reinforces the activation of myofibrillar proteins.

ANT1-TG animals express higher amounts of myofibrillar proteins, which stabilize the myocyte structure and contribute to a faster contraction. This is supposed to compensate for defects during heart failure. As evidenced, double transgenic ANT1/REN rats overexpressing ANT1 and renin, which normally leads to fulminant hypertension combined with cardiac hypertrophy, reveal an intact myocardial structure with a clear reduction of pathological changes such as apoptosis and fibrosis described for REN rats [6]. In addition, ANT1/REN rats exhibit left ventricular contractile reserve and hemodynamic compensation mechanisms that were sufficient to preserve cardiac function and prevent cardiac hypertrophy under hypertensive conditions. Furthermore, myocardial dysfunction, shown in heart failure animal models, is associated with reduced expression level and activity of SERCA2a [18, 34, 35] that can be restored in SERCA2a transgenic rats [13]. Thus, elevation of SERCA2a protein in ANT1-TGs in association with enhanced SERCA2a activity could compensate for diminished intracellular Ca^{2+} handling during heart failure.

In conclusion, our data clearly show that an ANT1 overexpression positively affects the contractile system by acceleration of cell contraction and relaxation. Alteration in ANT1 expression influences not only the mitochondrial function but also makes a significant impact on intracellular structural and functional processes. These functional changes in ANT1-TG animals remarkably contribute to accelerated cardiac performance and illustrate ANT's potency of cardioprotection during heart failure.

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