Altered function in atrium of transgenic mice overexpressing triadin 1

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Kirchhefer, Uwe, Hideo A. Baba, Yvonne M. Kobayashi, Larry R. Jones, Wilhelm Schmitz, and Joachim Neumann. Altered function in atrium of transgenic mice overexpressing triadin 1. Am J Physiol Heart Circ Physiol 283: H1334–H1343, 2002. First published May 30, 2002; 10.1152/ajpheart.00937.2001.—Triadin 1 is a protein in the cardiac junctional sarcoplasmic reticulum (SR) that interacts with the ryanodine receptor, junctin, and calsequestrin, proteins that are important for Ca2+ release. To better understand the role of triadin 1 in SR-Ca2+ release, we studied the time-dependent expression of SR proteins and contractility in atria of 3-, 6-, and 18-wk-old transgenic mice overexpressing canine cardiac triadin 1 under control of the α-myosin heavy chain (MHC) promoter. Three-week-old transgenic atria exhibited mild hypertrophy. Finally, atrial weight was increased by 110% in 18-wk-old transgenic mice. Triadin 1 overexpression was accompanied by time-dependent changes in the protein expression of the ryanodine receptor, junctin, and cardiac/slow-twitch muscle SR Ca2+-ATPase isofrom. Force of contraction was already decreased in 3-wk-old transgenic atria. The application of caffeine led to a positive inotropic effect in transgenic atria of 3-wk-old mice. Rest pauses resulted in an increased potentiation of force of contraction after restimulation in 3- and 6-wk-old mice and a reduced potentiation of force of contraction in 18-wk-old transgenic mice. Hence, triadin 1 overexpression triggered time-dependent alterations in SR protein expression, Ca2+ homeostasis, and contractility, indicating for the first time an inhibitory function of triadin 1 on SR-Ca2+ release in vivo.

protein expression; force of contraction; sarcoplasmic reticulum-calcium release

IN CARDIAC MUSCLE CELLS membrane depolarization leads to activation of voltage-sensitive sarcolemmal L-type Ca2+ channels resulting in Ca2+ entry into the cell. The size and duration of this initial Ca2+ trigger (28) determines the activation state of the Ca2+ release channel or ryanodine receptor, localized to the junctional sarcoplasmic reticulum. The activation of the ryanodine receptor initiates the release of stored Ca2+ from the sarcoplasmic reticulum (SR) into the cytosol.

This process, called Ca2+-induced Ca2+ release, leads to activation of the myofilaments and cardiac contractility. The removal of Ca2+ from the cytosol into the SR lumen by the ATP-dependent Ca2+ pump localized to the free SR allows muscle relaxation to occur.

The amount of Ca2+ released from the SR depends in part on a complex of proteins localized to the junctional SR membrane (37). This protein complex is composed of the following: 1) the ryanodine receptor or Ca2+ release channel itself; 2) calsequestrin, a Ca2+ storage protein located in the lumen of the SR; 3) junctin and triadin, which are transmembrane proteins localized to the junctional SR membrane, appear to anchor calsequestrin to the ryanodine receptor. Triadin was originally detected in SR membrane vesicles prepared from skeletal muscle (3, 13). On SDS-PAGE, skeletal muscle triadin was visible as a 95-kDa molecular mass protein. Skeletal muscle triadin is composed of a short NH2-terminal cytosolic domain, a single transmembrane domain, and large COOH-terminal domain located in the lumen of the SR (15, 16). The intraluminal domain, composed of a high density of positively and negatively charged amino acid residues, interacts with the ryanodine receptor and calsequestrin. Another junctional SR protein, junctin, is also highly charged and homologous to triadin and participates in the anchoring of calsequestrin to the ryanodine receptor.

Triadin 1 is the main isoform of triadin in cardiac muscle (18). It appears to be a splice variant of a single triadin gene (7, 18). Triadin 1 exhibits two mobility forms on SDS-PAGE, a 35- and a 40-kDa protein, which are the deglycosylated and glycosylated forms of the protein (18). The function of this glycosylation site is unclear. Both the skeletal muscle isoform of triadin and the cardiac triadin isoform (triadin 1) share virtually identical regions in the luminal domains that are highly enriched in charged amino acid residues. The positively and negatively charged amino acid residues lysine and glutamic acid are organized into KEKE motifs in this region and appear to stabilize β-strand
structures that may mediate the interaction with other proteins of the \(Ca^{2+}\) release complex in the junctional SR (17). The close proximity between triadin 1, the ryanodine receptor, calsequestrin, and junctin at the junctional SR membrane implies an important role for triadin 1 in cardiac excitation-contraction coupling.

Recent results with skeletal muscle triadin suggest that it is involved in \(Ca^{2+}\) release. Application of purified skeletal muscle triadin to membranes containing the ryanodine receptor inhibited \(Ca^{2+}\) channel activity (20, 31). Moreover, Groh et al. (5) demonstrated a decrease in the rate of \(Ca^{2+}\) release from the SR when antibodies were applied, which had been raised to amino acid residues 2–17 at the NH2-terminal cytoplasmic domain of skeletal muscle triadin. The diminished \(Ca^{2+}\) release was due to a decrease in the open probability of the channel.

In an effort to gain insight into the functional role of triadin in the heart, we generated transgenic mice overexpressing this protein in atrium and ventricle. The overexpression of canine cardiac triadin 1 to the ventricle of transgenic mice resulted in hypertrophy, prolonged \(Ca^{2+}\) transients, and an impaired mechanical relaxation (14). Effects of triadin overexpression on atrial function have not been reported to date. We asked whether overexpression of triadin 1 had similar or different biochemical and physiological sequelae in atrium compared with ventricle. We report that triadin 1 overexpression led to distinct time-dependent changes in the expression level of regulatory SR proteins, the SR-\(Ca^{2+}\) handling, the cellular architecture, and contractile parameters of transgenic atria of 3-, 6-, and 18-wk-old mice. In 3-wk-old transgenic atria, immunoblot analysis revealed a reduction only in the expression level of junctin. These atria exhibited a mild hypertrophy and the beginning of fibrosis. Interestingly, the force of contraction was diminished, although the application of 10 mM caffeine had a positive inotropic effect on force of contraction and stimulation pauses yielded a higher postrest potentiation. In addition to these results, in 6-wk-old transgenic atria the protein expression of the ryanodine receptor and cardiac/slow-twitch muscle SR \(Ca^{2+}\)-ATPase isoform (SERCA2a) was downregulated. In 18-wk-old transgenic atria, the expression levels of junctin, the ryanodine receptor, and SERCA2a were reduced drastically. These alterations were accompanied by a severe hypertrophy, fibrosis, and contractile failure. Furthermore, the postrest potentiation was now even lower in transgenic atria and the force-frequency relationship was changed. We conclude that the overexpression of triadin 1 in transgenic atria suppresses the SR-\(Ca^{2+}\) release in vivo, which leads consequently to an impaired \(Ca^{2+}\) homeostasis, histological alterations, and contractile failure.

**METHODS**

*Experimental animals.* Production of transgenic mice was described previously (18). Targeted overexpression of canine cardiac triadin 1 to mouse atrium and ventricle was achieved by subcloning the cDNA into a mouse cardiac a-myosin heavy chain promoter expression cassette (6). All experiments were performed on 3-, 6-, and 18-wk-old mice. Animals were handled and maintained according to approved protocols of the animal welfare committees of the University of Münster, Germany, and Indiana University.

**Histological examinations.** Wild-type and transgenic atria were immersed in 4% saline-buffered formaldehyde and embedded in paraffin. Longitudinal sections at a thickness of 5 \(\mu\)m were mounted on Silane-coated glass slides. Sections were stained with hematoxylin-eosin and Sirius red. For electron microscopy small pieces of left atrial tissue were fixed by immersion into 2.5% phosphate-buffered glutaraldehyde. The specimens were further fixed in buffered 1% osmium tetroxide for 2 h, dehydrated in graded ethanol series, and embedded in Epon. Ultrathin sections were stained with uranyl acetate and lead citrate. The sections were investigated under a Philips CM 10 transmission electron microscope.

**SDS-PAGE, immunoblotting, and antibodies.** Each individual atrium was homogenized at 4°C with a Polytron PT-10 (Kinematica; Lucerne, Switzerland) in 10 mM histidine, 0.25 M sucrose (pH 7.4), and then solubilized at room temperature in 7.5% SDS-buffer containing 62.5 mM Tris-HCl (pH 6.8), 5% glycerol, 5 mM dithiothreitol, and a trace of bromophenol blue. Protein (40 or 200 \(\mu\)g) samples for detection of the ryanodine receptor were separated on 8% or 5% (ryanodine receptor) SDS-PAGE, according to the method of Porzio and Pearson (33). After the proteins were transferred to nitrocellulose, the blots were incubated with different antibodies. The amounts of bound protein were detected by \(^{125}\text{I}^-\)-labeled protein A and quantified with the use of a PhosphorImager (Bio-Rad; Hercules, CA). Nitrocellulose membranes were incubated with different antibodies: the mouse monoclonal antibody 8G5 raised to canine triadin 1 (18), the polyclonal antibody TRN6 raised to residues 146–160 of mouse triadin (18), the mouse monoclonal antibody 1E9 raised against the ryanodine receptor SDS-PAGE, according to the method of Porzio and Pearson (33). After the proteins were transferred to nitrocellulose, the blots were incubated with different antibodies. The amounts of bound protein were detected by \(^{125}\text{I}^-\)-labeled protein A and quantified with the use of a PhosphorImager (Bio-Rad; Hercules, CA). Nitrocellulose membranes were incubated with different antibodies: the mouse monoclonal antibody 8G5 raised to canine triadin 1 (18), the polyclonal antibody TRN6 raised to residues 146–160 of mouse triadin (18), the mouse monoclonal antibody 1E9 raised against the ryanodine receptor type 2 (37), for junctin, the affinity-purified antibody JCN4 was produced in rabbits according to the protocol of Jones et al. (11), the mouse monoclonal antibody 2D12 raised against phospholamban (9), the mouse monoclonal antibody 2A7-A1 for detection of SERCA2a (11), and the affinity-purified rabbit antibody raised against canine cardiac calsequestrin (26). Protein concentrations were determined according to the method of Lowry et al. (24).

**Contractile function of isolated atrium.** Contractions of iso- metric left atria from wild-type and transgenic mouse hearts were measured as previously described by Neumann et al. (29). Atria were dissected under a modified oxygenated solution containing (in mM) 119.8 NaCl, 5.4 KCl, 1.8 CaCl2, 1.05 MgCl2, 0.42 NaH2PO4, 22.6 NaHCO3, 0.05 Na2EDTA, 0.28 ascorbic acid, and 5.5 glucose. The isolated atrial preparations were fixed with silk and then mounted on hooks of platinum wire in glass tissue chambers. Atria were incubated in the solution, which was continuously gassed with 95% O2-5% CO2, and maintained at 35°C. Muscles were stimulated with pulses of 5 ms duration and a voltage of 10–15% above threshold (Stimulator SD9, Grass; Quincy, MA). Force of contraction was measured with an isometric force transducer after each muscle was stretched over 30 min to the maximum of its length-tension relationship. Time to peak tension was calculated as time from 10% of peak contraction to peak contraction. Time of relaxation was calculated as time from peak force to 90% reduction of force. Force of contraction was measured before and 5 min after application of 10 mM caffeine. These atria were stimulated with 1 or 3 Hz during caffeine measurements as described in the appropriate legend.

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Force-frequency relationship and postrest potentiation. The force-frequency relationship and postrest potentiation experiments were measured on separate atrial preparations. In force-frequency relationship experiments, the frequency was stepped increased from 0.2 to 3 Hz (0.2, 0.4, 0.6, 1, 1.5, 2, and 3 Hz). Force of contraction was quantified after 5 min (steady-state conditions) at each frequency. We determined postrest potentiation at 1 and 3 Hz. Resting pauses were chosen from 1 to 300 s (1, 2.5, 5, 10, 15, 30, 45, 60, 120, 180, and 300 s). Data collection and calculation were made with BEMON version 2.1 software (Ingenieurbüro Jäckel; Hanau, Germany).

Materials. 125I-labeled protein A was obtained from DuPont-NEN (Boston, MA). All other chemicals were of reagent grade.

Statistical analysis. Data are reported as means ± SE. Statistical differences between the different types of mice were calculated by ANOVA, followed by Bonferroni’s t-test where appropriate. P < 0.05 was considered significant.

RESULTS

Triadin overexpression and Ca2+ regulatory proteins. Cardiac overexpression of triadin 1 in both ventricle and atrium was driven by the α-myosin heavy chain promoter (18). Crude homogenates were prepared from atria of 18-wk-old wild-type and transgenic mice, and probed with a canine triadin 1-specific mouse monoclonal antibody 8G5 (Fig. 1A, dog triadin 1). This antibody recognizes two protein mobility forms of triadin 1 (18). The lower 35-kDa and the upper 40-kDa bands represent the deglycosylated and glycosylated (Ψ) forms of triadin 1, respectively. Total triadin 1 (endogenous mouse triadin plus transgene related canine triadin) was measured with the polyclonal antibody TRN6 (Fig. 1A, total triadin 1). Total triadin 1 was overexpressed approximately sixfold in transgenic atria of all ages (Table 1). To test whether triadin 1 overexpression in transgenic atrium may affect other regulators of cardiac Ca2+ homeostasis, the expression levels of proteins located at the junctional or free SR were measured. Western blotting revealed that the level of junctin, a junctional SR protein, was markedly reduced by 67% in 3-wk-old transgenic atria (Table 1). However, the expression levels of all other regulatory SR proteins were unchanged at this time. In 6-wk-old transgenic atria, the protein expression of junctin and the ryanodine receptor was diminished by 71% (Table 1). In addition, the expression level of SERCA2a, a protein of the free SR, was slightly reduced by 26% (Table 1). The protein expression of phospholamban and calsequestrin was unchanged between transgenic and wild-type atria. The expression levels of junctin and the ryanodine receptor remained reduced by 86% and 60%, respectively, in 18-wk-old transgenic atria. The analysis of immunoblotting showed that the expression level of SERCA2a was even decreased by 65% in transgenic atria. In addition, the ratio of phospholamban/SERCA2a was unchanged in 3- and 6-wk-old and higher in 18-wk-old transgenic atria (Fig. 1B). Notably, the protein expression of junctin and the ryanodine receptor was also diminished, whereas SERCA2a was not downregulated in ventricular homogenates of adult transgenic mice (14).

Cardiac phenotype of transgenic mice and histological examination. The overexpression of triadin 1 was accompanied by hypertrophy in all periods measured as indicated by an increased weight of left atrium. The atrial weight was increased by 13%, 29%, and 110% in 3-, 6-, and 18-wk-old transgenic mice, respectively (Table 2). The body weight was not different between transgenic and wild-type mice of all ages (data not shown). The consequences of triadin 1 overexpression on cellular morphology were also assessed by histological examinations (Fig. 2). Sections stained with Sirius red revealed enhanced interstitial fibrosis in transgenic compared with wild-type atria (Fig. 2, A–F). The degree of fibrosis was 3.5%, 3.8%, and 24.9% in transgenic atria and 1.5%, 1.0%, and 2.3% in wild-type atria of 3- (Fig. 2, A and B), 6- (Fig. 2, C and D), and 18-wk-old (Fig. 2, E and F) mice, respectively. The severe hypertrophy in 18-wk-old transgenic atria was confirmed by stainings with hematoxylin-eosin (Fig. 2, G and H). Ultrastructural analysis (Fig. 2, I and J) demonstrated that cardiomyocytes from adult trans-
genic atria exhibited disorganization of myofilaments and mitochondria. Myofibrils were partially displaced by a coarse-grained electron-dense matrix (Fig. 2J). Furthermore, transgenic cardiomyocytes contained a number of membrane-limited vesicles. In contrast, overexpression of triadin 1 in adult mouse ventricle gave less hypertrophy (~16%) with no fibrosis. Electron microscopy of ventricular sections did not reveal any enlargement of SR vesicles (14).

**Force–frequency relationship.** The functional consequences of triadin 1 overexpression on contractile properties were investigated in electrically driven left atrial preparations. In atrial preparations from wild-type mice (Fig. 3, A–C), increasing the stimulation frequencies caused a decline in force of contraction, the so-called negative force-frequency relationship or the negative “Treppe” phenomenon (for a review, see Ref. 19). This phenomenon was observed in 3-, 6-, and 18-wk-old mice. Ventricle from wild-type mice exhibits in contrast a positive Treppe (14, 30).

A decrease of force of contraction in transgenic atria compared with wild-type atria occurred at low-stimulation frequencies between 0.2 to 0.6 Hz in 3-wk-old mice (Fig. 3A) and between 0.2 to 1.5 Hz in 6- and 18-wk-old mice (Fig. 3, A, B, and C). When the frequency was increased to 1 Hz or higher in 3-wk-old mice and 2 Hz or higher in 6- and 18-wk-old mice, force of contraction was almost comparable between both groups. However, one should note that force of contraction that we are plotting here absolute values of developed force. If one refers the force to the atrial weight (Table 2), the differences between transgenic and wild-type atria are even larger. Thus, one can extrapolate that per unit of myofilament, transgenic atria apparently generate much less force than wild-type atria. The decline in force of contraction under increased stimulation frequencies was comparable between transgenic and wild-type atria in 3- and 6-wk-old mice (Fig. 3, A and B), but lower in transgenic atria of 18-wk-old mice (Fig. 3C). Force of contraction declined by 41% in transgenic and by 66% in wild-type atria.

The time to peak tension was prolonged in 6- and 18-wk-old transgenic atria, and the time of relaxation was prolonged in transgenic atria of all ages at all stimulation frequencies tested. For example, at 1 Hz (Fig. 4), the time to peak tension was 27.5 ± 0.9, 28.1 ± 0.9, and 34.4 ± 1.5 ms in transgenic atria and 25.0 ± 1.3, 23.7 ± 0.8, and 27.5 ± 1.4 ms in wild-type atria of 3-, 6-, and 18-wk-old mice, respectively; the time of relaxation was 49.4 ± 2.2, 55.6 ± 2.4, and 65.0 ± 4.3 ms in transgenic atria and 41.3 ± 2.1, 43.1 ± 1.6, and 48.7 ± 1.3 ms in wild-type atria of 3-, 6-, and 18-wk-old mice, respectively.

**Response to caffeine.** The force of contraction was unchanged when 3- and 6-wk-old wild-type atria were stimulated at 1 Hz and exposed to 10 mM caffeine (Fig. 5). However, force of contraction was decreased by 77% in 18-wk-old wild-type atria. The negative inotropic effect of caffeine in adult wild-type atrium was reported by MacIntosh et al. (25). In contrast, transgenic atria of 3-wk-old mice exhibited a positive inotropic effect after caffeine exposure (Fig. 5). Force of contraction was increased by 35%. Furthermore, force of contraction was unchanged after caffeine exposure in 6-wk-old transgenic atria. In 18-wk-old transgenic atria, we measured a negative inotropic effect of caffeine. Force of contraction declined by 41%. Taken together, the force of contraction was always higher after caffeine application in transgenic atria. Similar results were obtained when force of contraction was measured after administration of 10 mM caffeine at 3 Hz (data not shown).

**Influence of stimulation frequency on postrest behavior.** At longer rest intervals more force was generated in wild-type atria of all ages (postrest potentiation). The postrest-dependent increase of force of contraction was affected by the initial stimulation rate (steady-state condition). The potentiation was much higher when the stimulation frequency was enhanced from 1 Hz (Fig. 6, A–C) to 3 Hz (Fig. 7, A–C). The degree of

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<th>3rd Wk Ratio (TRD/WT) Atrium</th>
<th>6th Wk Ratio (TRD/WT) Atrium</th>
<th>18th Wk Ratio (TRD/WT) Atrium</th>
<th>18th Wk Ratio (TRD/WT) Ventricle</th>
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<tr>
<td>Triadin 1</td>
<td>5.85*</td>
<td>6.14*</td>
<td>6.30*</td>
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<td>Junctin</td>
<td>0.33*</td>
<td>0.29*</td>
<td>0.14*</td>
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<td>Ryanodine receptor</td>
<td>0.85</td>
<td>0.85</td>
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<td>SERCA2a</td>
<td>0.79</td>
<td>0.74*</td>
<td>0.35*</td>
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<td>Calsequestrin</td>
<td>0.99</td>
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The levels of proteins in atrial homogenates of 3-, 6-, and 18-wk-old mice were determined as described in METHODS. Values for transgenic (TRD) atria are normalized to the values obtained for the wild-type (WT) group (n = 3–8). SERCA2a, cardiac/slow-twitch muscle sarcomplasmic reticulum (SR) Ca2+-ATPase isomorf. Data in the last column are from Ref. 14. *P < 0.05 vs. WT.

<table>
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<th>Atrial weight, mg, 3rd wk</th>
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<td>3.2 ± 0.1</td>
<td>3.6 ± 0.1*</td>
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<td>Atrial weight, mg, 6th wk</td>
<td>4.1 ± 0.4</td>
<td>5.3 ± 0.2*</td>
</tr>
<tr>
<td>Atrial weight, mg, 18th wk</td>
<td>4.2 ± 0.3</td>
<td>8.8 ± 1.0*</td>
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Left atrial weight was determined for 3-, 6-, and 18-wk-old WT and TRD mice. Values are means ± SE; n = 4–8 mice. *P < 0.05 vs. WT.
Potentiation was comparable at 1 Hz in wild-type atria of 3-, 6-, and 18-wk-old mice (Fig. 6, A–C). Furthermore, the degree of potentiation was lower at 3 Hz in wild-type atria of 18-wk-old mice (Fig. 7C) compared with younger mice (Fig. 7, A and B). In transgenic atria, postrest potentiation was also increased when stimulation frequency was changed from 1 Hz (Fig. 6, A–C) to 3 Hz (Fig. 7, A–C). This increase of postrest potentiation occurred in transgenic atria of all ages. Nevertheless, the relative increase (% of steady state) of force of contraction after rest was different between transgenic and wild-type atria in 3-, 6-, and 18-wk-old mice. The force of contraction was higher in transgenic atria of 3-wk-old mice at 1 Hz (Fig. 6A) and 3 Hz (Fig. 7A) at rest intervals from 5 to 60 s. In addition, force of contraction was higher in transgenic atria of 6-wk-old mice at 1 Hz at rest pauses from 10 to 60 s (Fig. 6B) and at 3 Hz from 5 to 45 s (Fig. 7B). Interestingly, force of contraction after rest was lower in transgenic atria at 1 Hz at rest intervals from 60 to 300 s (Fig. 6C), likewise at 3 Hz from 5 to 300 s (Fig. 7C). At 1 Hz, the maximal force of contraction was reached at 300 s in wild-type atria of all ages and at 120 or 60 s in transgenic atria of 3- and 6-, or 18-wk-old mice (Fig. 6, A–C). At 3 Hz, force of contraction was maximum at a rest interval of 180 or 120 s in wild-type atria of 3- and 6-,
DISCUSSION

The present study was done to examine the effects of triadin 1 overexpression in atrium. We have reported before that overexpression of triadin 1 in the ventricle was associated with downregulation of the ryanodine receptor (by 55%) and junctin (by 73%), whereas the protein expression of SERCA2a and phospholamban remained unchanged (14). Of note, these alterations in the expression of Ca\textsuperscript{2+} handling proteins of the junctional SR were accompanied by an increased intracellular diastolic Ca\textsuperscript{2+} level, cardiac hypertrophy (by 12%), and impaired relaxation in isolated ventricular cardiomyocytes and other ventricular preparations. Furthermore, there were frequency-dependent contractile abnormalities in ventricular cardiomyocytes (14). However, these studies were performed on ventricular preparations of adult mice (16- to 20-wk-old). Data on the effects of triadin 1 overexpression in atrium and their time course are still lacking. Here we studied the effects of triadin 1 overexpression in atrium of 3-, 6-, and 18-wk-old mice.

Atrium of young mice. In 3-wk-old transgenic atrium, immunoblot analysis revealed a large reduction in the expression level of junctin (by 67%), a protein localized to the junctional SR, which, at least in vitro, can bind to triadin 1 (11, 37). Junctin shares similarities in its structural organization and amino acid sequence with...
triadin 1 (7, 11). Hence, the downregulation of junctin in this early stage of atrial development may represent an adaptive mechanism to maintain a structural balance between the homologous proteins triadin 1 and junctin. In addition, our data further suggest that triadin 1 and junctin are functionally coupled in their role to modulate SR-Ca$^{2+}$ release. Recent in vitro studies revealed that triadin 1 might influence the open probability of the ryanodine receptor. The application of purified skeletal muscle triadin to the isolated ryanodine receptor inhibited the activity of the Ca$^{2+}$ release channel (20, 31). If triadin 1 acted as a potential inhibitor of the ryanodine receptor, one would expect a higher SR-Ca$^{2+}$ content in 3-wk-old mice. To test this possibility, we applied caffeine because it depletes completely the SR of Ca$^{2+}$. The released Ca$^{2+}$ would enter the cytosol, bind to troponin C and generate force of contraction in the isolated atrial preparation. Indeed, 10 mM caffeine developed a positive inotropic effect in transgenic atrial preparations, whereas force of con-

Fig. 6. Effects of triadin 1 overexpression on postrest potentiation. Influence of 1-Hz stimulation frequency on postrest behavior in atrial preparations of 3- (A), 6- (B), and 18-wk-old (C) WT and TRD mice. The force of contraction is shown as the percentage of steady state before rest. The rest periods were chosen from 1 to 300 s.

*$p < 0.05$ vs. WT

Fig. 7. Effects of triadin 1 overexpression on postrest potentiation. The stimulation frequency was set at 3 Hz. Force of contraction was measured before and after different rest pauses in the atria of 3- (A), 6- (B), and 18-wk-old (C) WT and TRD mice. Force of contraction is shown as the percentage of steady-state twitch tension. Rest periods were chosen from 1 to 300 s.

*$p < 0.05$ vs. WT
traction was unchanged in atrial preparations from 3-wk-old wild-type mice. In addition to the experiments with caffeine, rest-dependent changes in force of contraction have been used in different species to clarify the mechanisms of SR-Ca\(^{2+}\) cycling (1, 2, 23, 34). The higher relative increase in force of contraction in transgenic atrial preparations from 3-wk-old mice compared with age-matched wild-type preparations suggests that the net Ca\(^{2+}\) gain of the SR during rest periods is enhanced in transgenic atria. We suggest that triadin 1 operates as a potent in vivo inhibitor of SR-Ca\(^{2+}\) handling. The downregulation of the expression level of triadin 1 (36) in transgenic atria of 3-wk-old mice was accompanied by a downregulation of the expression of the free SR protein SERCA2a (by 26%). A diminished protein expression of SERCA2a and prolonged time parameters were observed in many models of hypertrophy and heart failure (8, 32).

**Atrium of 18-wk-old mice.** Overexpression of triadin 1 in 18-wk-old atria was accompanied by downregulation of junctin (by 86%) and the ryanodine receptor (by 60%), proteins of the junctional SR, and of SERCA2a (by 65%). The downregulation in the expression of junctional and free SR proteins was accompanied by alterations in the SR-Ca\(^{2+}\) handling, which were different from those in atria of 3-wk-old mice. Transgenic and wild-type atria exhibited a negative inotropic response to caffeine in contrast to younger mice. However, the SR-Ca\(^{2+}\) load (i.e., measured as the force of contraction after caffeine exposure) was still higher in 18-wk-old transgenic compared with wild-type atria. The negative inotropic response to caffeine under stimulation in both adult transgenic and wild-type atria may result from an impaired ability of the SR to accumulate Ca\(^{2+}\). This has been explained by an impaired translocation of Ca\(^{2+}\) to Ca\(^{2+}\) release sites in the presence of caffeine (4). An alternative explanation is that caffeine enhances the leak of Ca\(^{2+}\) from the SR (25).

The force of contraction was diminished from 0.2 to 1.5 Hz stimulation rates as we observed in 6-wk-old transgenic atria. The time parameters were prolonged in transgenic atria. Kadambi et al. (12) as well as our group (29) measured reduced rates of force development and of relaxation and prolonged times to peak tension and times of relaxation in phospholamban-overexpressing atrium. The phospholamban-SERCA2a ratio critically regulates both basal contraction and relaxation in mouse atrium (12). Therefore, the decreased expression of SERCA2a (i.e., resulting in a higher phospholamban-SERCA2a ratio) or a diminished expression of the ryanodine receptor (i.e., resulting in a lower content of Ca\(^{2+}\) release units) may contribute to the decreased force of contraction and prolonged time parameters in 18-wk-old transgenic atria. In addition, it is conceivable that the severe fibrosis in adult transgenic atria may impair cardiac contractility and relaxation. This may also explain the lower relative increase in force of contraction after rest pauses in transgenic atria of this age. The severe hypertrophy together with the fibrosis was observed in light microscopy and at the ultrastructural level. Hypertrophy and fibrosis developed from young to adult transgenic atria. It is conceivable that the time-dependent changes in the expression level of regulatory SR proteins, which were associated with an enhanced SR-Ca\(^{2+}\) content, led to the initiation of atrial hypertrophy. By analogy, calsequestrin-overexpressing myocardium exhibited similar alterations in the SR-Ca\(^{2+}\) handling and developed...
severe hypertrophy and contractile failure (10). In addition, an altered cytosolic Ca\(^{2+}\) content may act as an initial stimulus of these processes. An increased diastolic intracellular Ca\(^{2+}\) level was observed in ventricular cardiomyocytes overexpressing triadin 1 (14). High intracellular Ca\(^{2+}\) may result in the activation of several key phosphatases (e.g., calcineurin) or transcription factors (e.g., NFAT3 and MEF2) leading to cardiac hypertrophy (27).

In summary, the time-dependent overexpression of triadin 1 in atrium is associated with adaptive changes in the SR-Ca\(^{2+}\) content in transgenic atria. Over time, these alterations in the SR-Ca\(^{2+}\) handling are accompanied by (and may cause) hypertrophy, fibrosis, and impaired contractility.

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