Age-dependent biochemical and contractile properties in atrium of transgenic mice overexpressing junctin

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Junctin overexpression in the atrium of 3-, 6-, and 18-wk-old transgenic mice. The ratio of atrial weight and body weight was unchanged between junctin-overexpressing (JCN) and wild-type (WT) mice. The proportion of atrial weight to body weight was the same in 3-wk-old and 18-wk-old mice, whereas it was lower in 6-wk-old wild-type mice. The ratio of atrial weight to body weight was unchanged between junctin-overexpressing (JCN) and wild-type (WT) mice at all ages investigated (n = 6–8). The protein expression of triadin 1 was decreased starting in 3-wk-old JCN atria by 69%, whereas the expression of the ryanodine receptor was diminished in 6- (by 48%) and 18-wk-old (by 57%) JCN atria compared with age-matched WT atria. Force of contraction was decreased by 35% in 18-wk-old JCN atria compared with age-matched WT atria. In 3-wk-old JCN atria, the spontaneous beating rate of isolated right atria was higher than in 18-wk-old JCN mice compared with age-matched WT mice. Heart rate was lower by 9% in telemetry ECG recordings in 18-wk-old JCN mice during stress tests. The spontaneous beating rate of isolated right atria was higher than in 18-wk-old JCN mice compared with age-matched WT mice.

Junctin overexpression in ventricle affects the packing of ryanodine receptor subunits. The interaction of the ryanodine receptor with calsequestrin, triadin 1, and junctin, which have structural similarities, is mediated by at least two anchoring proteins, triadin 1 and junctin, which have structural similarities. The highly charged COOH-terminal regions of both proteins in the SR lumen are required for the stabilization of the interaction of junctin and triadin 1 with calsequestrin and the ryanodine receptor in vitro. The ryanodine receptor is a tetramer associated with FK-506 binding protein that stabilizes the interaction between individual ryanodine receptor subunits. Calsequestrin resides in the lumen of the junctional SR and stores Ca2+ for Ca2+ release with high capacity and low affinity. The highly charged COOH-terminal regions of both proteins in the SR lumen are required for the stabilization of the interaction of junctin and triadin 1 with calsequestrin and the ryanodine receptor in vitro.

Junctin is a 210-amino acid protein, running as a 26-kDa band on SDS-PAGE. Junctin is expressed in cardiomyocytes of the atrium and ventricle from several species. Junctin contains a cytoplasmic NH2-terminal segment, a single transmembrane segment (residues 23–44), and a SR intralumenal COOH-terminal segment (residues 45–210). Junctin seems to have several binding sites for calsequestrin. The interaction between junctin and calsequestrin depends strongly on the intraluminal SR Ca2+ concentration. Higher Ca2+ concentrations can reduce the strength of binding between both proteins. Junctin is thought to anchor to the ryanodine receptor at its second SR intraluminal loop. Junctin overexpression in ventricle affects the packing of calsequestrin within the junctional SR and facilitates the association of the junctional SR to the surface membrane of the t-tubules. The corbular SR, which is not associated with the sarcolemma, is defective regulation of SR Ca2+ release may contribute to the progression of heart failure. There is still little knowledge on the role of junctional SR proteins participating in SR Ca2+ release function besides the ryanodine receptor.

In cardiac muscle, the ryanodine receptor forms a quaternary protein complex with calsequestrin, triadin 1, and junctin, which are all located in the junctional SR. The ryanodine receptor is a tetramer associated with FK-506 binding protein that stabilizes the interaction between individual ryanodine receptor subunits. Calsequestrin resides in the lumen of the junctional SR and stores Ca2+ for Ca2+ release with high capacity and low affinity. The highly charged COOH-terminal regions of both proteins in the SR lumen are required for the stabilization of the interaction of junctin and triadin 1 with calsequestrin and the ryanodine receptor in vitro.
lacking junctin (37). Several in vitro studies reported a possible role of junctin in SR Ca\(^{2+}\) release of skeletal muscle (17, 18, 40). The first evidence for an involvement of junctin in the regulation of cardiac SR Ca\(^{2+}\) handling came from a transgenic model with progressive hypertrophy and heart failure. The overexpression of the β\(_1\)-adrenergic receptor in the heart was accompanied by a downregulation of junctin protein expression and altered Ca\(^{2+}\) transient kinetics (5). However, the physiological function of junctin in the heart remained speculative.

Therefore, we initiated a strategy in which junctin was overexpressed in myocardium of transgenic mice by use of the α-myosin heavy chain promoter (41). The exclusive transgenic overexpression of this junctional SR protein might help to identify the mechanisms of SR Ca\(^{2+}\) release in hearts with normal activity or with altered Ca\(^{2+}\) signaling. The 29-fold overexpression of junctin in the ventricle resulted in right heart failure, a higher peak current through the L-type Ca\(^{2+}\) channel, and bradycardia (10), whereas the 10-fold ventricular overexpression of junctin was associated with hypertrophy, lower SR Ca\(^{2+}\) content, and impaired relaxation (21). However, no time course was performed in both studies. Here, we focused our interest on the time-dependent study of the biochemical and contractile properties in the atrium of 3-, 6-, and 18-week-old 10-fold junctin-overexpressing (JCN) transgenic mice.

**METHODS**

**Experimental animals.** JCN mice were generated as described previously (41). Briefly, canine cardiac junctin cDNA was ligated into a mouse cardiac α-myosin heavy chain promoter expression cassette containing the SV40 transcriptional terminator (15). JCN mice were identified by standard PCR techniques. The transgenic lineage exhibited a 10-fold overexpression of the transgene in the atrium and ventricle. Hearts from 3-, 6-, and 18-week-old animals of both genders were used for experiments in accordance with guidelines of the institutes and the animal welfare committees of the University of Münster and Indiana University.

**Quantitative immunoblotting analysis.** Left atria from wild-type (WT) and JCN hearts were homogenized for 90 s at 4°C in 250 μl of 10 mM histidine and 0.25 M sucrose (pH 7.4) using a Polytron PT-10 (Kinematica; Lucerne, Switzerland) and stored frozen at −20°C in small aliquots. Protein concentration was determined by the method of Lowry et al. (26). Homogenate samples were separated by SDS-PAGE using an 8% polyacrylamide gel as described by Porzio and Pearson (34). Proteins were transferred to nitrocellulose membranes and probed with antibodies: the mouse monoclonal antibody SDB8 raised to purified recombinant canine junctin (41); the affinity-purified rabbit antibody Jcn4 raised to COOH-terminal residues 193–207 of mouse junctin (20); the mouse monoclonal anti-ryanodine receptor antibody 1E9 (42); the mouse monoclonal anti-SERCA2a antibody 2A7-A1 (16); the affinity-purified rabbit antibody to canine cardiac calsequestrin (28); and the affinity-purified rabbit antibody raised to residues 146–160 of mouse triadin (24). These antibodies were tested in several studies using either homogenates or membrane preparations (5, 18, 41). The amounts of bound protein were detected by 125I-labeled protein A and quantified using a Storm 860 (Molecular Dynamics; Sunnyvale, CA).

**Histological studies.** Atria from WT and JCN mice were fixed in 4% buffered formalin, dehydrated, and embedded in paraffin. Longitudinal tissue sections of 5 μm thickness were obtained from the left and right atrium. Paraffin sections were mounted on glass slides. Sections were stained with hematoxylin-eosin and Sirius red. The degree of fibrosis was quantified densitometrically with the use of Sirius red-stained sections.

**Measurement of contractile function in atrium.** Force of contraction was measured as previously described (32). Hearts from WT and JCN mice were excised, rinsed in a Tyrode buffer, and weighed. The bathing solution was composed of (in mM) 119.8 NaCl, 5.4 KCl, 1.8 CaCl\(_2\), 1.05 MgCl\(_2\), 0.42 NaH\(_2\)PO\(_4\), 22.6 NaHCO\(_3\), 0.05 Na\(_2\)EDTA, 0.28 ascorbic acid, and 5.05 glucose (pH 7.4). It was continuously gassed with 95% O\(_2\) and 5% CO\(_2\). Thereafter, left and right atria were dissected from isolated mouse hearts and weighed. The atria were attached with sutures of silk to a hook of platinum wire in the organ bath and an isotonic force transducer. Atria were incubated in the gassed Tyrode buffer at 35°C. After each muscle was stretched to the maximum of its length-tension relationship, left atria were stimulated as indicated with pulses of 5-sms duration and a voltage of 10–15% above threshold (Grass stimulator SD9; Quincy, MA). Input signals were amplified and continuously monitored on a chart recorder (FMI; Egelsbach, Germany). Contractile parameters were evaluated. Force of contraction was the difference between maximal and minimal developed tension at constant muscle length. 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 after the application of 10 mmol/l caffeine at a stimulation frequency of 1 Hz. The maximal effect of caffeine was determined. In addition, force of contraction was measured during 10 ms pulses (ECa\(_{150}\)) was cumulatively applied to the organ bath. [Ca\(_{150}\)] was increased from 1.8 up to 9 mmol/l. Each dose was applied for 5 min. Atria were stimulated with 1 Hz during the dose-response curve with CaCl\(_2\). Right atria were used for measurement of spontaneous beating rate.

**Force-frequency relationship, postrest behavior, and postrest recovery.** First, stimulation frequency was stepwise increased from 0.2 up to 3 Hz (0.2, 0.4, 0.6, 1, 1.5, 2, and 3 Hz). Force of contraction was determined after 5 min under steady-state conditions at each frequency. Thereafter, postrest potentiation was measured at 1-Hz stimulation frequency. Force of contraction was analyzed at the first beat after restimulation at resting pulses of 2.5, 5, 10, 15, 30, 45, 60, 120, 180, and 300 s. The recovery of steady state was assessed after a rest period of 120 s for the next 10 beats. Data sampling and analysis were ensured by REMON 2.1 software (Ingenieurbüro Jakel; Hanau, Germany).

**ECG recordings in sedated and freely moving mice.** We recorded sixlead surface ECGs in sedated mice of all ages (30 mg/kg ketamine-10 mg/kg xylazine applied intraperitoneally). To record an ECG in freely moving, nonsedated mice, we implanted telemetric ECG transmitters intraperitoneally into 18-wk-old WT and JCN mice to continuously record lead II of the surface ECG. The details of this technique have been described previously (22, 23). After a 10-day postoperative recovery period, telemetric ECGs were simultaneously and continuously recorded from pairs of mice during 24 h of normal activity and during two defined stress tests. These included 5 min of swimming in a water-filled cage (water temperature 34 ± 2°C, tank size 20 × 30 cm width, 15 cm depth) (22). On the following day, repetitive warm air jets were applied to the mice while they were in their cages performing normal activity. This protocol consisted of 10 consecutive air jet periods (15-s duration) alternating with 45 s without air jet (14, 23). This “mental stress” procedure resulted in repetitive increases in heart rate (23) and increases systemic blood pressure and sympathetic nerve activity in mice (14). All stress test protocols were performed simultaneously in matched pairs of mice. The ECG was continuously recorded for 60 min before the stress test, during each stress protocol, and for 55 min (swimming) or 50 min (warm air jets) after the end of the protocol. All ECGs were scrutinized for arrhythmias. Heart rate was compared during 5-min periods of normal activity, during the two exercise protocols, and during the last 5 min of the postexercise recording. PQ intervals were determined in beats of equal heart rate at low (500 beats/min) and high heart rates (750 beats/min). All analyses were performed using a custom-written ECG analysis software programmed in LabView.
Materials. 125I-labeled protein A was obtained from DuPont-New England Nuclear (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

Overexpression of junctin in atrium and SR proteins. Transgenic mice overexpressing canine junctin in the atrium were engineered by use of the α-myosin heavy chain promoter (7). For the following experiments, we used transgenic mice with a 10-fold cardiac-specific overexpression of junctin (41). To test the expression of the transgene, we separated atrial homogenates from WT and JCN mice by SDS-PAGE and probed with the mouse monoclonal antibody 5D8, which specifically binds to canine junctin. The antibody detected canine junctin at a comparable expression level in atria of 3-, 6-, and 18-wk-old JCN mice (Fig. 1, dog junctin). Junctin ran as a 26-kDa molecular mass protein. In addition, we tested the protein expression level of triadin, the ryanodine receptor, and calsequestrin, proteins which are all located at the junctional SR. The rabbit polyclonal antibody TRN6 detected two protein mobility forms of triadin 1 (Fig. 1). It ran as a doublet of 35- and 40-kDa molecular mass proteins, corresponding to the deglycosylated and glycosylated (Ψ) forms of triadin 1, respectively (24). The expression level of triadin 1 was reduced by 69%, 88%, and 82% in 3-, 6-, and 18-wk-old JCN atria, respectively (Fig. 1 and Table 1). Of note, junctin was the protein most decreased in atrial homogenates of triadin 1 overexpressing (TRD) mice (19). In contrast, the protein expression of the ryanodine receptor was unchanged between JCN and WT atria of 3-wk-old mice (Fig. 1 and Table 1). However, the expression level of the ryanodine receptor was downregulated by 48% and 57% in 6- and 18-wk-old JCN atria, respectively (Fig. 1 and Table 1). Remarkably, the protein expression of the ryanodine receptor also decreased constantly with rising age in TRD atria (19). The protein expression of calsequestrin, the main Ca2+ storage protein in the lumen of the junctional SR, was comparable between JCN and WT atria in mice of all ages (Fig. 1 and Table 1). Moreover, the protein expression of sarco(endoplasmic reticulum Ca2+-ATPase 2a (SERCA2a), the Ca2+ pump of the free SR, remained unchanged between JCN and WT atria of 3-, 6-, and 18-wk-old mice (Fig. 1 and Table 1).

Basal and [Ca2+]o-dependent contractile parameters in transgenic atria. To determine the mechanical consequences of junctin overexpression, we measured contractile parameters in isolated atrial tissue preparations. Under basal conditions with 1.8 mmol/l [Ca2+]o, the force of contraction was unchanged between 3- and 6-wk-old JCN and WT atria (Fig. 2, A and B), and force of contraction was decreased by 35% in 18-wk-old JCN atria (Fig. 2C). However, the decrease of force of contraction in adult JCN mice was not accompanied by morphological or structural alterations of these atria. The ratio of atrial weight to body weight was not different between adult JCN and WT mice as well as in young mice (Table 2). Furthermore, 18-wk-old JCN atria were not affected by a reduction of the contractile tissue or fibrosis (Table 2). In contrast, adult TRD atria exhibited severe hypertrophy (i.e., atrial weight was increased by 110%) and interstitial fibrosis (19). Here, we determined [Ca2+]o-dependent force of contraction in atria of 3-, 6-, and 18-wk-old mice. Force of contraction increased for [Ca2+]o up to 5.4 mmol/l in WT atria of all ages investigated (Fig. 2). Force of contraction increased even up to 9 mmol/l [Ca2+]o in 3-wk-old JCN atria (Fig. 2A), whereas the force development was comparable at various [Ca2+]o between JCN and WT atria of 6-wk-old mice (Fig. 2B). Finally, the net effect of increasing [Ca2+]o on force of contraction was higher in 18-wk-old JCN than in WT atria, because the basal force was diminished (Fig. 2C). Under basal contractile conditions (1.8 mmol/l [Ca2+]o), the overexpression of junctin had no effect on the time to peak tension in atria of all ages studied (Fig. 3). Whereas the time of relaxation was unchanged between 3- and 6-wk-old JCN and WT atria, time of relaxation was slightly but significantly prolonged by 9% in adult JCN atria (Fig. 3).

Effects of stimulation frequency on developed force. The WT mouse atrium exhibits a decline of force of contraction at higher stimulation frequencies (‘negative staircase phenome-
Here, this negative force-frequency relationship was detected in young and adult WT atrium. Interestingly, the decline of developed force at higher stimulation frequencies was diminished in 18-wk-old TRD atria (19). On the basis of those data, we tested the force-frequency relationship in atria of JCN mice. However, for all ages studied, the decline of force of contraction at higher stimulation frequencies (from 0.2 to 3 Hz) was similar in JCN and WT atria of 3-, 6-, and 18-wk-old mice (data not shown).

**Beating rate of isolated right atria and determination of heart rate in mice with ECG recording.** In addition to the study of the force-frequency relationship on left atria, we measured the rate of isolated, spontaneously beating right atria. The beating rate decreased constantly from young to adult WT atria (Fig. 4). Whereas the beating rate was similar in JCN and WT atria of 3- and 6-wk-old mice, the beating rate was higher in 18-wk-old JCN right atria (Fig. 4). The beating rates of isolated right atria were compared with in vivo heart rates recorded with surface ECG or telemetric ECG. In anesthetized mice, heart rates were not different between JCN and WT mice of all ages investigated (data not shown). Likewise, in freely moving mice, heart rate was not different between 18-wk-old JCN and WT mice during normal activity and during recovery from exercise (Table 3). During mental stress, heart rate was lower in JCN mice. A similar trend was observed during swimming exercise ($P = 0.18$), consistent with a decreased chronotropic response to exercise. PQ intervals were not different at heart rates of 750 beats/min during exercise and at heart rates of 500 beats/min during normal activity between JCN and WT mice (Table 3).

**Postrest potentiation and recovery of steady state after restimulation.** The overexpression of junctin resulted in an altered stoichiometry of the junctional SR proteins, including

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### Table 2. Morphometric parameters

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<thead>
<tr>
<th>Age</th>
<th>WT</th>
<th>JCN</th>
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<tbody>
<tr>
<td>3 wk old</td>
<td>0.23 ± 0.01</td>
<td>0.24 ± 0.01</td>
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<tr>
<td>6 wk old</td>
<td>0.19 ± 0.01</td>
<td>0.16 ± 0.01</td>
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<tr>
<td>18 wk old</td>
<td>0.14 ± 0.01</td>
<td>0.17 ± 0.01</td>
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Fibrosis, %

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<tr>
<th>Age</th>
<th>JCN</th>
<th>WT</th>
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<tbody>
<tr>
<td>18 wk old</td>
<td>0.97</td>
<td>0.75</td>
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Values are means ± SE; $n = 6–8$ atria. The ratio of left atrial weight (AW) and body weight (BW) was determined for 3-, 6-, and 18-wk-old WT and JCN mice. The degree of fibrosis in transgenic and WT atrium is included.

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Fig. 2. Effects of extracellular Ca$^{2+}$ ($[\text{Ca}^2+]_o$) on force of contraction. Force of contraction was measured in WT and JCN atria of 3- (A), 6- (B), and 18-wk-old mice (C) in response to increasing concentrations of $[\text{Ca}^2+]_o$ at 1-Hz stimulation frequency. Alterations were detected in 3- and 18-wk-old JCN atria.

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Fig. 3. Contractile time parameters. Time to peak tension (TPT) and time of relaxation (TOR) were determined (in ms) in WT and JCN atria of 3-, 6-, and 18-wk-old mice. The stimulation frequency was adjusted to 1 Hz.
the ryanodine receptor, triadin, and calsequestrin. Thus we would expect changes in SR Ca\(^{2+}\) content and/or SR Ca\(^{2+}\) release in JCN atrium under non-steady-state conditions. This was addressed by experiments on atria in which phases of rest were initiated, followed by phases of restimulation. The application of rest pauses from 2.5 to 300 s resulted in an increase of developed force after restimulation in WT atria of 3-, 6-, and 18-wk-old mice (Fig. 5). This increase in the contraction amplitude after rest periods is termed postrest potentiation. In addition, the degree of potentiation was comparable in young and adult WT atria. In JCN atria, we observed a potentiation of force of contraction at all rest pauses in young and adult mice (Fig. 5). However, the relative increase of force of contraction after restimulation was higher in JCN atria of 3-wk-old mice (Fig. 5A) at rest intervals from 30 to 300 s. Furthermore, force of contraction after rest was not different between JCN and WT atria of 6- (Fig. 5B) and 18-wk-old mice (Fig. 5C). Figure 6 shows representative traces of single experiments to assess the contractile behavior under non-steady-state conditions of JCN and WT atria of 3-wk-old mice. The degree of potentiation was comparable between both groups at the 5-s rest period, whereas potentiation was higher in JCN atria when 120-s rest pauses were chosen. The recovery of steady state after rest periods can be used as a first estimation of the extent of refilling or emptying of the SR with Ca\(^{2+}\). The steady state of twitches

<table>
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<th>Table 3. Telemetric ECG recordings</th>
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<td>HR, beats/min</td>
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<tr>
<td>Normal activity</td>
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<tr>
<td>Swimming</td>
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<tr>
<td>Warm air jet</td>
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<td>Postswimming</td>
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<td>Postwarm air jet</td>
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<td>PQ interval, ms</td>
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<td>At 750 beats/min</td>
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<td>At 500 beats/min</td>
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<tr>
<td>Values are means ± SE; n = 5 atria. Telemetric ECGs were continuously recorded in 18-wk-old WT and JCN mice during 48 h of normal activity and defined stress tests (see METHODS). The PQ intervals were determined at different heart rates (HRs). *P &lt; 0.05 vs. WT.</td>
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was reached 8–10 beats upon resumption of stimulation after 120-s rest in JCN and WT atria of all ages (Fig. 7). However, the relative decay of developed force back to steady state was higher during the first beats in 3-wk-old JCN atria (Fig. 7A). In addition, the initial recovery of twitches (i.e., the restoring of SR Ca\textsuperscript{2+} after restimulation was unchanged between JCN and WT atria of 6- (Fig. 7B) and 18-wk-old mice (Fig. 7C).

**Caffeine application.** Caffeine has been used in many species and cardiac preparations to induce muscle contractures. The amplitude of the caffeine-induced contractures has been used indirectly as an index of the stored Ca\textsuperscript{2+}/H\textsubscript{11001 in the SR. Here, we measured the maximal effect of 10 mmol/l caffeine on force of contraction. The maximum was reached 15–30 s after application of 10 mM caffeine. This concentration is sufficient to induce SR Ca\textsuperscript{2+} release in atria. Caffeine exhibited a positive inotropic effect in 6-wk-old WT and 3- and 6-wk-old JCN atria (Fig. 8). Force of contraction was higher after caffeine application in 3-wk-old JCN compared with WT atria (Fig. 8).

**DISCUSSION**

Here, we characterized the age-dependent functional consequences of junctin overexpression in the mouse atrium. The study of the effects of junctin overexpression in the atrium is of special interest because atria overexpressing triadin 1 exhibited multiple alterations in structural morphology and excitation-contraction coupling (19). In earlier work, the overexpression of triadin 1 was also studied in the atrium from 3-, 6-, and 18-wk-old mice, thus allowing comparison of age-dependent effects of both transgenes. Here, we found that the overexpression of junctin in the atrium exhibited both similarities and differences in age-dependent functional effects compared with triadin 1 overexpression in the atrium. The results will be discussed on the background of our previously published data on TRD animals (19). For clarity, the results of the present and previous study are summarized in Table 4.

**Altered SR Ca\textsuperscript{2+} handling in 3-wk-old JCN atrium.** The protein expression of triadin 1 was downregulated in JCN atria. In comparison, junctin was reduced in the atrium with triadin 1 overexpression of 3-wk-old mice (Fig. 9 and Table 4). The expression levels of the ryanodine receptor and calsequestrin remained unchanged in JCN and TRD atria at this age (Table 4). This observation shows that the downregulation of the homologous proteins junctin and triadin is an early mechanism of adaptation. In principle, a reduced protein expression of triadin 1 can occur either from primary at present unknown alterations in the gene expression, causing a decreased protein synthesis, or from a reduced half-life of the protein or from an enhanced protein degradation. The latter seems obvious because junctin reaches the junctional SR directly after synthesis.
as revealed by electron microscopy studies (38). Thus, during trafficking of junctin to the junctional SR, the overexpressed junctin may displace triadin 1 from the quaternary complex, causing the protein to be more rapidly degraded. In contrast to junctin, calsequestrin appears to be delivered to the junctional SR by a Golgi-mediated mechanism (38). Furthermore, the caffeine-induced developed force and the potentiation of force of contraction after restimulation were increased both in 3-wk-old JCN and TRD (19) atria, suggesting a higher SR Ca$^{2+}$ content (Fig. 9 and Table 4). This may result, in part, from a tighter packing of calsequestrin in proximity of the ryanodine receptor in JCN hearts (38). As a consequence, the retained calsequestrin may provide more activatable Ca$^{2+}$ to the ryanodine receptor for its release. However, a postrest potentiation of developed force occurred also in rat ventricular cardiomyocytes without any increase in SR Ca$^{2+}$ content as assessed by caffeine administration (1). These authors made a rest-dependent increase in fractional SR Ca$^{2+}$ release responsible for the postrest potentiation. In addition, one should take into consideration that the application of caffeine was performed on multicellular atrial tissue preparations in our study, which is critical in terms of the interpretation of the results (2). Moreover, JCN atria of 3-wk-old mice developed more force under high [Ca]o (9 mmol/l). Although we did not measure the global Ca$^{2+}$ transient in isolated atrial cardiomyocytes, it is possible that the higher contractile response to extracellular Ca$^{2+}$ in 3-wk-old JCN atria was maintained by efficient Ca$^{2+}$ removal systems. This can be attributed to a higher Ca$^{2+}$ extrusion via the Na$^{+}$/Ca$^{2+}$ exchanger and/or enhanced Ca$^{2+}$ uptake into the SR. However, the protein expression of SERCA2a was unchanged in 3-wk-old JCN atria. Finally, the relative decay of force of contraction during the first beats after restimulation was higher in 3-wk-old JCN atria, which may result from a stimulation-dependent decrease in fractional SR Ca$^{2+}$ release.

**Table 4. JCN versus TRD atria**

<table>
<thead>
<tr>
<th></th>
<th>3 wk old</th>
<th>6 wk old</th>
<th>18 wk old</th>
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<tr>
<td></td>
<td>JCN</td>
<td>TRD</td>
<td>JCN</td>
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<tr>
<td>Junctin</td>
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<tr>
<td>Triadin</td>
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<td>Ryanodine receptor</td>
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<td>Calsequestrin</td>
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<td>SERCA2a</td>
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<td>Atrial weight</td>
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<tr>
<td>Degree of fibrosis</td>
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<td>Basal force of contraction, 1 Hz</td>
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<td>Time of relaxation</td>
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<tr>
<td>Decay of force-frequency relationship</td>
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<tr>
<td>Postrest potentiation, 1 Hz</td>
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<tr>
<td>Caffeine-induced force of contraction</td>
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</table>

TRD, triadin overexpressing; ↔, no change; ↑, mild increase; ↑↑, strong increase; ↓, mild decrease; ↓↓, strong decrease.

**Downregulation of junctional SR proteins in 6-wk-old JCN atria.** In our study, we found a large downregulation of triadin 1 (by 88%) in JCN atria of 6-wk-old mice. Moreover, the expression level of the ryanodine receptor was reduced in JCN atria. A comparable situation was observed in atria with triadin 1 overexpression of 6-wk-old mice. The homologous protein to triadin 1, junctin, was downregulated, and we detected a decreased expression of the ryanodine receptor (Table 4). In addition, the expression of calsequestrin was unchanged both in atria with junctin and triadin 1 overexpression (Table 4). It is possible that the downregulation of triadin 1 or junctin (depending on the model) and the ryanodine receptor may facilitate the normal interaction of the proteins of this quaternary complex. Although we detected these similarities in the expression of junctional SR proteins in JCN and TRD atria, we failed to detect alterations of the functional parameters studied in JCN atria. In contrast, the overexpression of triadin 1 in 6-wk-old atria was accompanied by hypertrophy, decreased contractility, prolonged time parameters, higher postrest potentiation, and increased caffeine-induced force of contraction (Table 4). It remains to be elucidated why 6-wk-old JCN atria exhibited a “normalization” of SR Ca$^{2+}$ handling parameters (e.g., postrest potentiation).

**Depressed contractility in 18-wk-old JCN atria.** Here, 18-wk-old JCN atria developed less force under basal conditions. The downregulated expression of the ryanodine receptor may contribute to the decreased contractility. It is likely that, besides a reduced expression of the ryanodine receptor, the downregulation of SERCA2a may contribute to the contractile failure in TRD atria (Fig. 9 and Table 4). Furthermore, the rate of spontaneously beating right atria was increased in adult JCN mice, whereas the heart rates in the intact mice, recorded with surface or telemetric ECG, were unchanged between both groups during normal activity. This discrepancy may result from a diminished sympathetic tonus in intact JCN mice. To further determine whether JCN mice respond adequately to stress (i.e., β-adrenergic receptor stimulation by catecholamines), we determined the heart rates during swimming exercise and warm air jets by telemetric ECG recordings (23). The JCN mice exhibited a decreased chronotropic response to mental stress. Recently, a possible role for SR Ca$^{2+}$ release in sinoatrial node pacemaker activity during basal conditions and β-adrenergic receptor stimulation has been suggested (11, 35).
This is supported by the fact that the application of isoproterenol after the inhibition of the ryanodine receptor in rabbit sinoatrial nodal cells failed to increase the diastolic depolarization rate and the spontaneous firing rate (39). Thus the increase of the firing rate of sinoatrial nodal cells in response to β-adrenergic receptor stimulation requires an intact function of the ryanodine receptor. Thus we suggest that the reduced protein expression of the ryanodine receptor in JCN mice may contribute to a lower recruitment of intralumenal SR Ca\(^{2+}\) during stress resulting in a decreased chronotropic response. In addition, the force-frequency relationship, the postrest behavior, and the response of developed force to caffeine were comparable between atria of 18-wk-old JCN and WT mice, whereas all these parameters were altered in adult atria with triadin 1 overexpression (Table 4). Although the SR Ca\(^{2+}\) content was increased in TRD atria, we found a diminished postrest potentiation suggesting a diastolic SR Ca\(^{2+}\) leak. This may explain, in part, the prolongation of the time of relaxation in TRD atria. One possible explanation for differences observed in these two transgenic models is that the overexpression of triadin 1 modulates the interaction of the junctional SR proteins and therefore the SR Ca\(^{2+}\) handling more potently than the overexpression of junctin. Furthermore, it is conceivable that the downregulation of junctin in TRD atria is more critical for the regulation of SR Ca\(^{2+}\) release. Consistently, the reduced protein expression of junctin in mice overexpressing the β\(_{1}\)-adrenergic receptor was associated with altered SR Ca\(^{2+}\) handling (5). The comparison of the biochemical and physiological effects in JCN and TRD atria revealed another interesting aspect. Whereas we observed an unchanged atrial weight in JCN atria, a severe atrial hypertrophy, fibrosis, and ultrastructural abnormalities were detected in TRD atria (Table 4). We suggest that only triadin 1 overexpression may affect the kinetics of SR Ca\(^{2+}\) release. The slow, sustained Ca\(^{2+}\) (i.e.,
the higher diastolic SR Ca\(^{2+}\) leak) can activate the calcineurin-dependent regulatory pathway leading to the transcription of several genes (e.g., GATA-4) that are involved in the induction of cardiac hypertrophy (31). Furthermore, it is conceivable that Ca\(^{2+}\) “shuttles” via at present unknown mechanisms from the SR to the nucleus (13, 33). This Ca\(^{2+}\) may then induce directly the transcription of hypertrophy-associated genes in TRD but not in JCN mice (Fig. 9).

In summary, in 3-wk-old atria, the overexpression of junctin was associated with a decreased protein expression of triadin 1. This resulted in a higher SR Ca\(^{2+}\) content without changes in contractility or heart rate. In 6-wk-old JCN atria, the putative SR to the nucleus (13, 33). This Ca\(^{2+}\) may then induce directly the transcription of hypertrophy-associated genes in TRD but not in JCN mice (Fig. 9).

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