Altered expression of connexin43 contributes to the arrhythmogenic substrate during the development of heart failure in cardiomyopathic hamster

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Submitted 17 August 2007; accepted in final form 6 December 2007

Sato T, Ohkusa T, Honjo H, Suzuki S, Yoshida M, Ishiguro YS, Nakagawa H, Yamazaki M, Yano M, Kodama I, Matsuzaki M. Altered expression of connexin43 contributes to the arrhythmogenic substrate during the development of heart failure in cardiomyopathic hamster. Am J Physiol Heart Circ Physiol 294: H1164–H1173, 2008. First published December 7, 2007; doi:10.1152/ajpheart.00960.2007.—Heart failure is known to predispose to life-threatening ventricular tachyarrhythmias even before compromising the systemic circulation, but the underlying mechanism is not well understood. The aim of this study was to clarify the connexin43 (Cx43) gap junction remodeling and its potential role in the pathogenesis of arrhythmias during the development of heart failure. We investigated stage-dependent changes in Cx43 expression in UM-X7.1 cardiomyopathic hamster hearts and associated alterations in the electrophysiologic properties using a high-resolution optical mapping system. UM-X7.1 hamsters developed left ventricular (LV) hypertrophy by ages 6–10 wk and showed a moderate reduction in LV contractility at age 20 wk. Appreciable interstitial fibrosis was recognized at these stages. LV mRNA and protein levels of Cx43 in UM-X7.1 were unaffected at age 10 wk but significantly reduced at 20 wk. The expression level of Ser255-phosphorylated Cx43 in UM-X7.1 at age 20 wk was significantly greater than that in control golden hamsters at the same age. In UM-X7.1 at age 10 wk, almost normal LV conduction was preserved, whereas the dispersion of action potential duration was significantly increased. UM-X7.1 at age 20 wk showed significant reduction of cardiac space constant, significant decrease in conduction velocity, marked distortion of activation fronts, and pronounced increase in action potential duration dispersion. Programmed stimulation resulted in sustained ventricular tachycardia or fibrillation in UM-X7.1. LV activation during polymorphic ventricular tachycardia was characterized by multiple phase singularities or wavebreaks. During the development of heart failure in the cardiomyopathic hamster, alterations of Cx43 expression and phosphorylation in concert with interstitial fibrosis may create serious arrhythmogenic substrate through an inhibition of cell-to-cell coupling.

cardiomyopathy; ventricular arrhythmia; optical mapping; phosphorylation

LIFE-THREATENING VENTRICULAR tachyarrhythmias often occur in patients with heart failure even at a compensated stage (6). The underlying mechanisms are not well understood. In the heart, gap junctions provide the pathway for intercellular current flow, enabling coordinated action potential propagation and contraction. Gap junction channels are constructed from connexins (Cxs), a multigene family of conserved proteins. To date, the connexin (Cx) gene family comprises 20 members in the mouse and 21 members in the human genome. In the mammalian heart, the following Cxs have been identified: Cx37, Cx40, Cx43, and Cx45 (30). In adult ventricular muscle, cell-to-cell coupling is provided predominantly by Cx43 channels and to a lower extent by Cx45 (11, 19, 31). Newly synthesized Cx43 disappears rapidly with a half-life of only 2.5–5.0 h, and its function is regulated by phosphorylation (30). Consequently, dynamic turnover of Cxs in the heart may be an important mechanism modulating intercellular coupling and impulse propagation. There are several phosphorylation sites in Cx43, and the functional consequence of phosphorylation is variable. The phosphorylation of Cx43 on Ser255 has been shown to cause gap junctional uncoupling in cultured cell lines (40). In many recent studies (4, 5, 8, 9, 15, 19, 20, 27, 29, 31, 38, 39, 43, 44), altered expression, organization, and phosphorylation of the Cx43 in ventricular muscle has been demonstrated in human patients as well as animal models of diverse heart diseases, including ischemic, hypertrophic, and tachycardia-induced cardiomyopathy, and genetically engineered animals. Such gap junction remodeling is supposed to contribute to the abnormal conduction properties and arrhythmias in the ventricle under these pathological conditions. As to idiopathic cardiomyopathy, however, the available information is still limited and much remains to be clarified. The present study was designed to shed light on this issue.

We used a strain of the Syrian cardiomyopathic hamster, UM-X7.1 (a derivative of the BIO14.6 strain), since its characteristic features of heart failure progression have been demonstrated in previous studies (36, 37); the animals show moderate compensated left ventricular (LV) contractile dysfunction at ages 10–20 wk and serious decompensated heart failure at ages beyond ~24 wk. In the present study, we investigated stage-dependent changes in Cx43 expression in UM-X7.1 at ages 6–20 wk. Associated alterations in the electrophysiologic properties and propensities for life-threatening arrhythmias were also investigated using a high-resolution optical mapping system. The results reveal that UM-X7.1 hamsters at age 20 wk, compared with golden hamsters (controls), exhibit a significant reduction in Cx43 expression (mRNA and protein) and moderate interstitial fibrosis. Interestingly, the proportion of...
the Ser255-phosphorylated form of Cx43 is increased compared with control animals at the same age. This gap junction remodeling is accompanied by reduced electrical cell-to-cell coupling, serious distortions in conductivity, repolarization inhomogeneity, and an increased propensity for ventricular tachycardia/fibrillation (VT/VF).

**MATERIAL AND METHODS**

**Animal model.** UM-X7.1 cardiomyopathic hamsters (kindly provided by Dr. Lemanskie, SUNY Health Science Center, Syracuse, NY, and inbred in our laboratory) and sex- and age-matched normal golden hamsters (control, Japan SLC, Hamamatsu, Japan) were used as experimental animals. All animal procedures were approved by the Yamaguchi University Experimental Animal Care and Use Committee. The investigation conforms with the Guide for Care and Use of Laboratory Animals published by the US National Institute of Health (NIH Publication No. 85-23, revised 1996).

**Echocardiography, electrocardiography, and histology.** Echocardiography was performed using an echocardiograph model SSD-1000, with a 10-MHz sector scan probe (Aloka, Tokyo, Japan). Left ventricular end-diastolic diameter (LVEDd), left ventricular end-systolic diameter (LVESd), and fractional shortening (FS) were calculated according to a standard formula, as applied in our previous studies (36, 37). ECGs were recorded using a PowerLab System (AD Instruments, Colorado Springs, CO) for 24 h.

Specimens for histological examination were obtained from heart cross slices cut mid-way between the apse and base. The samples were embedded in paraffin, and 4-μm thick sections were cut and stained. The connective-tissue volume-fraction was assessed with the aid of Azan staining and calculated as previously described (37).

**RT-PCR amplification.** Total ventricular RNA was isolated from frozen cell samples by the acid guanidinium thiocyanate/phenol/chloroform extraction method (25). cDNA was prepared using a Takara RNA PCR kit (Takara, Tokyo, Japan; Ref. 26). The primers were based on rat sequences: sense primer, 5'-CAT TGA CCT CAA CTA CAT GGT-3'; antisense primer, 5'-GCA ATC GAG TGG CGT CTG GCT GCTCAA-3'. For GAPDH, they were based on rat sequences (GenBank accession no. AY206455): sense primer, 5'-GGG CTG CTA AGA ACC TAC ATC ATC AGT-3'; antisense primer, 5'-CCA TGA CCT CAA CTA CAT GGT-3'. The GAPDH gene product was used as an internal control, since it is still used widely in RT-PCR amplification. The background fluorescence was subtracted from each frame to reveal the signal. The fluorescence signals were inverted and then averaged to reduce noise. Spatial resolution after the long-pass filtering was 0.18–0.24 mm. Isochrone maps were generated from the filtered grayscale data. To analyze action potential configuration, a five-point time median filter was applied to the spatially averaged data (23), and then the data were normalized to within the range of the maximum and the minimum values in the respective 1,000 frames sampled. A point at 10% depolarization in the upstream phase and a time point at 90% repolarization were identified, and their interval was measured as the action potential duration at 90% repolarization (APD90). The dispersion of APD90 values in the recording area was displayed as color gradient maps with 1.0-ms steps ranging from red (the shortest) to blue (the longest). The pattern of wave propagation during VT/VF was quantified using the phase mapping method described by Gray et al. (13). Phase singularity (PS) was defined as the point at which all phases converged.

Conduction velocity (CV) was measured during constant stimulation (S1) at the center of the LV free wall at a basic cycle length of 200 ms. The CV was measured in a central 6 × 6-mm square around the stimulation site, as measurement in the outer periphery would be hampered by the sharp curvature of the ventricular surface. The longitudinal (L) direction of propagation was determined from the activation map so that it crossed the most widely spaced isochrones. A second line for transverse (T) direction of propagation was drawn perpendicular to the first line through the densely spaced isochrone. The CVs in the L (CVL) and T (CVT) directions were calculated from the slope of a linear least-square fit of the activation time plotted against the distance. Data from an area very close to the stimulation site (<1.0 mm) were excluded to minimize the virtual electrode polarization effects.

APD and its distribution were measured under similar constant stimulation at the apex. The pulses applied were 2.0 ms in duration at an intensity 1.3 times the threshold. We used a premature stimulation...
protocol to induce VT/VF. After rapid stimulation at a S1-S1 interval of 150 ms, a single premature stimulation (S2) was applied at the center of the anterior LV free wall, and the sequence was repeated by progressive shortening of the S1-S2 interval. Widely spaced bipolar electrogams were recorded between the apex of the LV and the high lateral wall of the RV to monitor the whole ventricular excitation.

Measurement of intercellular electrical coupling. Intercellular electrical coupling was assessed by optically measuring overall tissue resistivity in the LV of Langendorff-perfused hearts according to the methods originally described by Akar et al. (3): spatial decay of transmembrane potential in response to subthreshold depolarizing stimuli was measured with the use of the high-resolution optical mapping system, and the space constant (λ) was determined by fitting of the data with a single exponential function. In these experiments, magnified fluorescence images (covering 5 × 5 mm, 256 × 256 pixels) were obtained through a photographic lens with a longer focal distance (Micro-Nikkor 105 mm f/2.8D, Nikon) at a sampling rate of 0.5 Hz. A single cathodal subthreshold stimulus (20 ms in duration, ~0.8 times threshold) was delivered during electrical diastole after regular (250- to 400-ms intervals) suprathreshold stimuli was delivered with the use of the high-resolution optical mapping system, and the space constant (λ) was determined by fitting of the data with a single exponential function. In these experiments, magnified fluorescence images (covering 5 × 5 mm, 256 × 256 pixels) were obtained through a photographic lens with a longer focal distance (Micro-Nikkor 105 mm f/2.8D, Nikon) at a sampling rate of 0.5 Hz. A single cathodal subthreshold stimulus (20 ms in duration, ~0.8 times threshold) was delivered during electrical diastole after regular (250- to 400-ms intervals) suprathreshold stimuli (2.0 ms in duration, ~1.2 times diastolic threshold) through a Teflon-coated platinum electrode (100 μm in diameter) placed at the center of the anterior LV free wall. To induce subthreshold membrane potential responses to such long depolarizing stimuli, myocardial excitability was reduced by an increase in the extracellular K+ concentration from 5 to 8 mmol/l. Membrane responses to subthreshold stimuli were estimated as the maximum change in fluorescence intensity relative to the resting level and normalized with respect to the amplitude of baseline action potential signals at each recording site.

Statistics. All data are presented means ± SD. Comparisons between data were made by ANOVA. Frequency of VT/VF induction was compared with χ²-test analysis. Differences were taken to be significant at P < 0.05.

RESULTS

Somatic and cardiac growth. The body weight of UM-X7.1 hamsters was significantly smaller at each stage than that of golden hamsters; however, the value of the LV-to-body weight ratio was significantly higher in each UM-X7.1 group (Fig. 1, A and B, n = 5 in each group). Mean cardiomyocyte width of UM-X7.1 hamsters was significantly greater than that in golden hamsters at ages 10–24 wk (Fig. 1C, n = 5 in each group). The connective-tissue volume-fraction of UM-X7.1 hamsters was significantly higher than that of golden hamsters (Fig. 1, D and E, n = 5 in each group; 5.3 ± 0.5 vs. 1.0 ± 0.2% at age 10 wk, 7.0 ± 0.5 vs. 1.9 ± 1.1% at age 20 wk, and 12.8 ± 1.5 vs. 2.5 ± 0.8% at age 24 wk).

Echocardiography and electrocardiography. Figure 2A shows representative M-mode echocardiograms of the LV at the papillary muscle level in each group of hamsters. Table 1 shows serial changes in LV dimensions and FS in UM-X7.1 and golden hamster groups (n = 5 in each group). At age 20 wk, LVEDd and LVESd were significantly greater and FS was significantly lower in UM-X7.1 hamsters than those in golden hamsters. Figure 2B shows representative ECGs for each group. A significant prolongation in QRS and PQ duration was detected in UM-X7.1 hamsters at ages 20 and 24 wk (Fig. 2B, n = 5 in each group). These observations indicate that UM-X7.1 hamsters develop cardiac hypertrophy with interstitial fibrosis at age 10 wk and suffered from cardiac dysfunction with ECG abnormality at age 20 wk and thereafter. As shown in Fig. 2C (Kaplan-Meier survival curve), UM-X7.1 hamsters died from congestive heart failure at over age 25 wk. Based on these results, we focused on the gap junction remodeling and its consequent electrophysiological changes during the compensated stage (ages 10–20 wk) of heart failure in UM-X7.1 cardiomyopathic hamsters.

Cx43 expression. To quantify Cx43 expression (mRNA and protein), RT-PCR and Western blot analysis were performed. Cx43 expression (mRNA and protein) in UM-X7.1 hamsters at age 20 wk, compared with golden hamsters, was significantly reduced by 33 and 55%, respectively (Fig. 3, A and B, n = 5 in each group). The total content of Cx45 in UM-X7.1 hamsters at age 20 wk did not show any difference from control hamsters (Fig. 3C, n = 5 in each group). Quantitative immunoconfocal microscopy revealed that Cx43 positive area decreased significantly in UM-X7.1 hamsters at age 10 wk (by 33%) and the reduction was more prominent at age 20 wk (by 58%) compared with golden hamsters at the corresponding age.
Table 1. *Echo geometry findings and cardiac function*

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<th>Golden</th>
<th>UM-X7.1</th>
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<tr>
<td></td>
<td>6 wk</td>
<td>10 wk</td>
</tr>
<tr>
<td>LVEDd, mm</td>
<td>4.5±0.3</td>
<td>4.8±0.1</td>
</tr>
<tr>
<td>LVESd, mm</td>
<td>3.0±0.2</td>
<td>3.1±0.2</td>
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<tr>
<td>FS, %</td>
<td>33±5</td>
<td>34±2</td>
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Values are means ± SD (n = 5 in each group). LVEDd, left ventricular end-diastolic diameter; LVESd, left ventricular end-systolic diameter; FS, fractional shortening. *P < 0.01 vs. 6 wk of the same strain; †P < 0.05; ‡P < 0.01 vs. golden hamster.

(Fig. 4, n = 5 in each group). Interestingly, the relative expression level of Ser255-phosphorylated Cx43, which has been shown in the mouse cell line to cause the downregulation of gap junctional intercellular communication (40), was markedly increased in UM-X7.1 hamsters compared with golden hamsters at age 6 wk (1.55 ± 0.19 vs. 0.96 ± 0.30, P < 0.01) and age 20 wk (0.83 ± 0.34 vs. 0.28 ± 0.29, P < 0.01; Fig. 5A, n = 5 in each group). The relative expression level of Tyr265-phosphorylated Cx43 showed no significant difference between UM-X7.1 and golden hamsters at ages 6–20 wk (Fig. 5B, n = 5 in each group).

Figure 5C shows the double staining for total Cx43 (green) and Ser255-phosphorylated Cx43 (red). In LV from golden hamsters at age 20 wk, staining for total Cx43 (green) and Ser255-phosphorylated Cx43 (red) was recognized at the cell termini. The Cx43 staining patterns of LV tissue from UM-X7.1 hamsters at age 20 wk were markedly different from those of golden hamsters; total Cx43 staining (green) at cell termini was decreased, whereas Ser255-phosphorylated Cx43 immunolabeling was increased at cell termini (red). Positive staining for Ser255-phosphorylated Cx43 was also recognized in cytosolic areas (red). Merged images showed yellow-colored staining at the cell termini. Lateralization of Cx43 did not appear in UM-X7.1 hamsters at ages 6–20 wk.

Electrophysiological properties and vulnerability for VT/VF. Conduction properties were examined during constant stimulation (S1) from a center of the LV free wall. The isochrones of the activation front exhibited a smooth, symmetric, elliptical pattern in golden hamsters at ages 10 and 20 wk (Fig. 6A, left; the online version of this article contains supplemental data; see Supplemental Movie 1); the axis of the ellipse corresponded to the fiber orientation of the subepicardial cardiac muscle (data not shown). In the central 6 × 6-mm square, there was a linear correlation between activation time and distance in both the L and T directions. CV_L and CV_T in golden hamsters at age 10 wk were 43.9 ± 0.6 and 17.4 ± 2.3 cm/s, respectively (n = 5 in each group). The anisotropic ratio ([CV_L/CVT] = 2.6 ± 0.5. The correlation coefficient (CC) between activation time and distance was 0.98–0.99 (Table 2). Comparable values were obtained for these parameters of conduction for golden hamsters at age 20 wk, and there were no significant age-dependent changes. In UM-X7.1 hamsters at age 10 wk, the isochrones of the activation front also showed a smooth, symmetric elliptical pattern, and all the parameters of the conduction properties (CV_L, CV_T, CV_L/CVT, and CCs) were similar to those of golden hamsters (Table 2). In UM-X7.1 hamsters at age 20 wk, however, the isochrones of activation front were extremely distorted, indicating spatial inhomogeneity of propagation (Fig. 6A, right; see Supplemental Movie 2). Both CV_L and CV_T were significantly decreased by 35–40% compared with golden hamsters at age 20 wk (Table 2). Although CV_L/CVT was comparable, CCs, which reflect the spatial homogeneity of propagation, were significantly decreased in UM-X7.1 (Table 2).
Figure 6A, top, shows color maps of APD90 during constant stimulation from the apex. The APD90 values in the entire mapping area are displayed as color gradients with the shortest APD90 colored red and the longest colored blue. Figure 6A, bottom, shows superimposed optical action potential signals recorded from 16 sites covering a 6 × 6-mm square. In golden hamsters at both ages 10 and 20 wk, the APD90 maps were homogeneously colored red with a minimal dispersion of APD90, and there were no appreciable age-dependent changes (Fig. 6B; Table 2). In UM-X7.1 hamsters, the averaged APD90 was unaffected at age 10 wk, whereas it was prolonged at 20 wk compared with golden hamsters. The dispersion of APD90 in UM-X7.1 was significantly larger than golden hamsters even at age 10 wk, reflected by a mixture of red and blue (Fig. 6B). The APD dispersion in UM-X7.1 was more pronounced at age 20 wk (Table 2).

Induction of VT/VF was attempted by a premature stimulation protocol. After 19 basic rapid stimulations (S1-S1 150 ms), a single premature stimulus (S2) was applied with progressive shortening of the S1-S2 interval. In golden hamsters (n = 10 and 11 at ages 10 and 20 wk, respectively), no arrhythmias were induced. In UM-X7.1, in contrast, VT or VF was induced in 3 of 11 hamsters at age 10 wk (27%, P = 0.075 vs. golden at age 10 wk) and 6 of 6 hamsters at age 20 wk (100%, P < 0.0001 vs. golden at age 20 wk; P < 0.005 vs. UM-X7.1 at age 10 wk; Table 3). Optical images of VT/VF episodes lasting >5 s were recorded in four UM-X7.1 hearts at age 20 wk. Representative data are shown in Fig. 7. Polymorphic VT (PVT) was induced in one UM-X7.1 hamster at age 20 wk, whereas no arrhythmias were induced in a same age golden hamster (Fig. 7A). Figure 7B shows six sequential phase maps at the initiation of PVT (see Supplemental Movie 3). A PS of counterclockwise rotation (white circle) appeared in the upper middle region of the LV (133 ms after S2) and moved back and forth in the middle region (208–258 ms). A second PS of clockwise rotation (black circle) appeared in the upper right region at 258 ms and stayed there until the end of the data sampling (500 ms) with minimal meandering. A third PS of counterclockwise rotation coming from the upper left margin (294 ms) collided with the first PS, resulting in their dissipation (mutual annihilation) at 337 ms. A fourth PS of clockwise rotation and a fifth PS of counterclockwise rotation appeared near the apex at 389 and 448 ms, respectively, constructing a figure eight reentry circuit. The distance between the two PSs initially increased and then decreased, culminating in mutual annihilation (461 ms). In addition to these multiple PSs, multiple wavebreaks of incomplete rotation appeared frequently during the PVT episode (black arrowheads at 208 and 294 ms). Complex movements of the five PSs are illustrated in Fig. 7C. Figure 7D shows the change in PS number during the PVT episode (from 100–500 ms after S2). The average number of
PSs during the 400 ms was 1.92. Qualitatively similar complex activation patterns with multiple PSs and wavebreaks were observed in phase maps of the remaining VT/VF episodes.

**Intercellular electrical coupling.** Application of subthreshold chathodal unipolar stimuli resulted in instantaneous membrane depolarization spatially localized in the vicinity of the stimulus electrode (Fig. 8A, left, and B), whereas suprathreshold stimuli induced propagating action potentials (Fig. 8A, right). The amplitude of membrane depolarization in response to subthreshold stimuli was decreased in space almost exponentially with increasing distances from the site of stimulation (Fig. 8, C and D). The λ estimated from the spatial decay of the subthreshold membrane depolarization varied along and across the myocardial fiber orientation (λ_L and λ_T, respectively, in Fig. 8D), reflecting a normal uniform anisotropic architecture of ventricular myocardium. Representative values of λ_L and λ_T obtained from a golden hamster at age 20 wk (Fig. 8D) were comparable to those reported for adult guinea pig ventricular myocardium (3). The difference between control and UM-X7.1 hamsters was analyzed only for λ_T, because precise evaluation of λ_L was often hampered by small hyperpolarization of membrane potential 1- to 2-mm distant in the direction along the fiber orientation from the stimulation site when relatively large subthreshold stimuli were applied. This may be the result of virtual

**Fig. 5.** Phosphorylated Cx43 expression (n = 5 in each group). A: Ser255-phosphorylated Cx43 expression. Amount of Ser255-phosphorylated Cx43 was normalized to the value of GAPDH protein. *P < 0.01 vs. 6 wk of the same strain; #P < 0.01 vs. age-matched golden hamster. B: Tyr265-phosphorylated Cx43 expression. Amount of Tyr265-phosphorylated Cx43 was normalized to the value of GAPDH protein. Open bars: golden hamsters; closed bars: UM-X7.1 hamsters. All data were normalized to the value of an age 6-wk golden hamster, which was set to a value 1. C: representative double staining for total Cx43 (green), Ser255-phosphorylated Cx43 (red), and merged (yellow). In age 20-wk golden hamster LV, total Cx43 staining mainly exists at the cell termini. In age 20-wk UM-X7.1 hamster, total Cx43 staining at cell termini was decreased, and Ser255-phosphorylated Cx43 immunolabeling increased at cell termini (arrow) and in the cytoplasmic area (arrowhead). Arrow indicates Ser255-phosphorylated Cx43.

**Fig. 6.** Conduction properties and action potential configurations in golden and UM-X7.1 hamsters at ages 10 and 20 wk. A: isochrone maps of activation during constant stimulation [basic cycle length (BCL) = 200 ms]; 1.0-ms intervals for golden at ages 10 wk and 20 wk and UM-X7.1 at age 10 wk and 2.0-ms intervals for UM-X7.1 at age 20 wk. A dotted square (6 × 6 mm) surrounds the area for measurements of conduction velocity. L, longitudinal direction; T, transverse direction. Bidirectional arrow indicates the subepicardial myocardial fiber orientation confirmed in the histological section (hematoxylin & eosin) cut parallel to the epicardial surface. B: action potential duration at 90% repolarization (APD_{90}) and its distribution on the anterior surface of LV. Top: maps of APD_{90} in the recording area are displayed as color gradients in 1-ms steps ranging from red (shortest) to blue (longest). Records were obtained during constant stimulation (BCL = 200 ms) from the apex (st). Bottom: superimposed action potential signals recorded at 16 sites (white dots on top).
Table 2. Conduction properties and action potential configurations

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<th>Golden</th>
<th>UM-X7.1</th>
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<tr>
<td></td>
<td>10 wk</td>
<td>20 wk</td>
</tr>
<tr>
<td>CVL, cm/s</td>
<td>43.9±0.6</td>
<td>43.6±1.1</td>
</tr>
<tr>
<td>CVT, cm/s</td>
<td>17.4±2.3</td>
<td>17.4±1.5</td>
</tr>
<tr>
<td>CVI/CVT</td>
<td>2.6±0.5</td>
<td>2.5±0.2</td>
</tr>
<tr>
<td>CC_L</td>
<td>0.99±0.01</td>
<td>0.99±0.01</td>
</tr>
<tr>
<td>CC_T</td>
<td>0.98±0.00</td>
<td>0.98±0.01</td>
</tr>
<tr>
<td>APD90, ms</td>
<td>84±4</td>
<td>78±6</td>
</tr>
<tr>
<td>APDD, ms</td>
<td>11.0±2.0</td>
<td>10.0±5.5</td>
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Values are means ± SD (n = 5 in each group). CVL, conduction velocity in the longitudinal direction; CVT, conduction velocity in the transverse direction; CVI/CVT, anisotropic ratio of conduction velocity; CC_L, correlation coefficient between activation time and distances for the measurement of CVL; CC_T, correlation coefficient between activation time and distances for the measurement of CVT; APD90, action potential duration at 90% repolarization (average of values at 16 sites); APDD, APD90 dispersion among 16 sites.

DISCUSSION

The novel finding of this study is that UM-X7.1 cardiomyopathic hamsters at a compensated stage of heart failure show a ~55% reduction in the amount of Cx43 protein and abnormal phosphorylation in the ventricular myocardium in addition to mild interstitial fibrosis. These changes were associated with a decrease in cardiac \( \lambda_L \), an inhibition of conduction, an increased dispersion of repolarization, and an enhanced propensity to VT/VF resulting from multiple reentrant circuits with complex meandering. In UM-X7.1 hamsters, quantitative and qualitative alterations of Cx43 in concert with interstitial fibrosis may therefore create serious arrhythmogenic substrates through an inhibition of cell-to-cell coupling.

**Downregulation of Cx43 in failing hearts.** It is now well recognized that conduction disturbance in the failing heart is attributed at least in part to disturbances of gap junction organization. The most consistently observed alteration in ventricular Cx expression involves downregulation of Cx43 in patients with end-stage heart failure due to idiopathic dilated cardiomyopathy or ischemic heart disease (31). Downregulation of Cx43 was also demonstrated in several animal models. In dog hearts with pacing-induced heart failure, Poelzing and Rosenbaum (28) reported that a substantial reduction of immunoreactive Cx43 (by 40% in average) was accompanied by a significant reduction in intercellular coupling and CV in association with an enhancement of transmural dispersion of APD. In a study using similar dog model of heart failure, Akar et al. (5) showed not only a quantitative reduction of Cx43 protein expression but also redistribution of Cx43 from the intercalated disk region to lateral cell borders and relative increase of hypophosphorylated fraction of Cx43. In a transgenic mouse model of juvenile dilated cardiomyopathy, Hall et al. (16) showed that a reduction of Cx43 expression and conduction defect become apparent long before development of severe congestive heart failure. The present study using the cardiomyopathic hamster UM-X7.1 is in line with their study in terms of Cx43 downregulation during the development of heart failure.

In our cardiomyopathic hamster model, although immunofluorescence data revealed significant decrease in Cx43 at age 10 wk (hypertrophic stage; Fig. 4), the total content of Cx43 estimated by Western blotting showed no significant Cx43 reduction compared with golden hamsters at the same age (Fig. 3B). A possible explanation for this inconsistency may be translocation of Cx43 from the surface membrane to the cytosol, which is detected by Western blot.

In failing hearts, activation of several neurohumoral factors (e.g., rennin-angiotensin-aldosterone system, cytokines, and catecholamines) could affect synthesis, assembly, disassembly, degradation, and phosphorylation of connexins. The downregulation and altered phosphorylation of Cx43 in UM-X7.1 hamster hearts could be reversed by inhibitors of such neurohumoral factors. These issues were not studied in the present experiments. Phosphorylation of Cx43 on Ser255 by p34^cdc2 kinase in Rat1 cells was shown to promote endocytosis and degradation (22). It seems reasonable to speculate that translocation and degradation of Cx43 in cardiac cells might be the result of phosphorylation of specific residues.

**Alterations in Cx43 as arrhythmogenic substrates.** The relationship between the quantitative changes in Cx43 expression and conduction properties of the cardiac impulse has been studied in a variety of genetically engineered mouse models. Data on conduction properties in heterozygous knockout (KO) mice (~50% reduction of Cx43), which develop normally and have normal lifespan, are contradictory; some investigators reported 23–44% conduction slowing (12, 14), whereas others detected no significant conduction abnormalities (24). Gutstein et al. (15) demonstrated in cardiac-restricted Cx43 KO mice that a marked reduction of Cx43 (by 95%) resulted in a significant reduction in CV (by 42–55%) and a high incidence of spontaneous lethal ventricular tachyarrhythmias. The same group studied the quantitative relationship of Cx43 downregulation, conduction disturbance, and ventricular arrhythmia inducibility by using a subline of Cx43 KO mice with progressively decreasing levels of Cx43 at older ages (9). The results revealed that an ~80% reduction of Cx43 is required for the genesis of serious arrhythmogenic conduction disturbance.

In the present study with the use of UM-X7.1 hamsters, a moderate reduction of Cx43 (by 55% at age 20 wk) was
associated with significant reduction of CV (by 35–40%), an increased dispersion of ventricular repolarization, and a high susceptibility to VT/VF induction. This suggests some additional factors to compromise the normal uniform conduction in the cardiomyopathic hamster.

An age-dependent progression of fibrosis (from ages ~10 wk) may be most important among these factors. According to the cable theory, the CV is inversely proportional to the square root of tissue resistivity, which is composed of intracellular (Ri) and extracellular (Ro) resistances (21). Interstitial fibrosis is expected to cause an increase of Ro and if it is combined with an increase of Ri resulting from Cx43 downregulation, the net effect to compromise conduction would be much greater than the increase of Ri alone. Our experiments to measure spatial decay of electrotonic depolarization in response to subthreshold stimuli showed that effective λ, which is also inversely proportional to the square root of tissue resistivity (Ri + Ro) in the cable theory, was in fact significantly less in UM-X7.1 at age 20 wk (by 32%) compared with golden hamsters at the same age. Spach et al. (32) have demonstrated in their simulation study that cellular scaling (cell size) plays an important role in determining anisotropic conduction properties by affecting the cell-to-cell delay of activation and the maximum upstroke velocity. In UM-X7.1 hamsters at age 10 wk, the
anisotropic conduction properties were unaffected despite of significant increase in cell size. This apparent discrepancy might be due to a relatively small cell hypertrophy (an increase of cell width by ~25%) in the UM-X7.1 hamster compared with the simulation by Spach et al. (~4-fold increase in cell surface area). Concomitant moderate increase (~5%) of connective tissue (fibrosis) in the UM-X7.1 hamster might have different effects on the anisotropic conduction properties to make the issue more complex.

The second factor to be considered is phosphorylation of gap junction proteins affecting the channel conductance, trafficking, and/or the assembly or disassembly (22, 30). Rapid de-phosphorylation and translocation of Cx43 from the intercalated disk region have been reported in rat hearts subjected to acute ischemia (7). Dephosphorylation of Cx43 and inhibition of intercellular communication have also been reported in animal models of nonischemic heart failure (1, 5). On the other hand, the phosphorylation of specific serine sites (e.g., Ser368 by PKC and Ser255, Ser279, and Ser282 by MAPK) or a tyrosine site (e.g., Tyr265 by Src) in Cx43 was reported to cause a rapid attenuation of gap junctional communication (22). Toyofuku et al. (34) demonstrated in BIO14.6 cardiomyopathic hamster hearts that the level of Tyr265-phosphorylated Cx43 was increased at the advanced stage of heart failure and that the change was accompanied by an increase in c-Src activity and a reduction in gap junctional communication.

In the present study, despite the reduction in total Cx43 expression, the expression of Ser255-phosphorylated Cx43 was significantly increased in UM-X7.1 at age 20 wk compared with golden hamsters at the same age. In contrast, there was no significant difference in the expression level of Tyr265-phosphorylated Cx43 between the two animal groups. Interestingly, Ser255-phosphorylated Cx43 was upregulated not only at the intercalated disc region but also in the cytosolic area. Based on these observations, it seems reasonable to speculate that relative increase of dysfunctional or nonfunctional Ser255-phosphorylated Cx43 gap junctions in UM-X7.1 may contribute as well to the inhibition of cell-to-cell electrical coupling in the heart.

The present results showed that there was a marked age-dependent reduction of Ser255-phosphorylated Cx43 in golden hamsters and that the age-dependent change was attenuated in UM-X7.1. The pathophysiological meaning of this observation remains unclear, and further experimental studies will be required to elucidate the issue.

The third factor to be addressed is potential changes in other Cx members. Yamada et al. (41) reported upregulation of Cx45 in conjunction with downregulation of Cx43 in the end stage of human failing ventricles. In the present study using UM-X7.1 hamsters, there was no significant difference in the level of Cx45 expression between the cardiomyopathic and control hamsters at ages 6~20 wk. This result is in line with the study using BIO14.6 cardiomyopathic hamster hearts (34).

Study Limitations

There are several limitations in the present study. First, we estimated electrical cell coupling by measuring overall tissue resistivity. With the use of a high-resolution optical mapping system, spatial decay of transmembrane potential in response to subthreshold depolarizing stimuli was measured, and the $\lambda$ was determined by fitting of the data with a single exponential function according to the method originally described by Akar et al. (3). This is not a direct measure of cell-to-cell coupling, since the tissue resistivity is composed of both $R_i$ and $R_c$, in the cable theory and does not allow distinction between the two parameters. A better direct method to quantify loss of coupling might be to look at individual cell pairs using either current or dye injection. It was, however, practically difficult to isolate ventricular cell pairs, which are suitable for the measurements, from UM-X7.1 hamsters at age 20 wk by enzymatic digestion, because of a plenty of connective tissues and more fragile cell membranes in the pathological condition. In addition, mammalian cardiac tissue is best modeled by bidomain equations with unequal anisotropic ratios of intracellular and extracellular domains rather than a simplified one-dimensional cable equation (21). According to the two or three-dimensional bidomain model, membrane depolarization during unipolar current injection does not decay as a simple exponential function from a point of current injection (21). In optical measurement experiments using Langendorff-perfused guinea pig hearts, however, Akar et al. (3) have demonstrated that there is a close linear relationship between $\lambda$ and the CV at different angles with respect to the fiber orientation and that pharmacological intercellular uncoupling decreases $\lambda$ and the CV in parallel. These observations support the feasibility of $\lambda$ as a useful approximate index of cardiac cell coupling.

Second, we did not investigate remodeling of other ion channels, including the sarcolemmal Na+, Ca2+, and K+ channels, which are responsible for depolarization and repolarization of action potentials. Altered expression and function of these ion channels in UM-X7.1 hamsters could contribute to the conduction disturbance and increased dispersion of repolarization in favor of serious reentrant ventricular arrhythmias.

Third, we could not get spontaneous monitoring ECG records of lethal ventricular arrhythmias (PVT/VF) resulting in sudden death in the UM-X7.1 hamsters. Hano et al. (17) reported a high propensity for ventricular arrhythmias in cardiomyopathic hamsters with no signs of heart failure.

Fourth, we could not measure the transmural heterogeneities of cellular repolarization and their potential role in heart failure-related arrhythmias in our mapping system with small animal models. Transmural electrophysiological heterogeneities are known to be an important substrate of arrhythmogenesis in heart failure (4). Finally, our findings are certainly of relevance to hamster models but not necessarily of human idiopathic cardiomyopathy condition specifically.

ACKNOWLEDGMENTS

This study was supported by in part by a grant-in-aid for scientific research in Japan (C17590738 and C19590818) from the Ministry of Education, Japan.

REFERENCES


