Cell Coupling Between Ventricular Myocyte Pairs From Connexin43-Deficient Murine Hearts

Jian-An Yao,* David E. Gutstein,* Fangyu Liu, Glenn I. Fishman, Andrew L. Wit

Abstract—Mice with cardiac-restricted inactivation of the connexin43 gene (CKO mice) have moderate slowing of ventricular conduction and lethal arrhythmias. Mechanisms through which propagation is maintained in the absence of Cx43 are unknown. We evaluated gap junctional conductance in CKO ventricular pairs using dual patch clamp methods. Junctional coupling was reduced to 4±2 nS (side-to-side) and 11±2 nS (end-to-end), including 21% of cell-pairs with no detectable coupling, compared with 588±104 nS (side-to-side) and 558±92 nS (end-to-end) in control cell-pairs. Voltage dependence of control gap junctions was characteristic of Cx43. CKO conductance showed increased voltage dependence, suggesting low-level expression of other connexin isoforms. From theoretical models, this degree of CKO coupling is not expected to support levels of conduction persisting in vivo, suggesting the possibility that there are additional mechanisms for maintained propagation when gap junctional conductance is severely reduced. (Circ Res. 2003;93:736-743.)

Key Words: gap junctions ■ connexin ■ remodeling ■ arrhythmias

Changes in gap junction structure and function in pathological conditions (gap junction remodeling) may contribute to arrhythmogenesis.2-9 Gap junction protein quantity is reduced in heart failure, cardiomyopathy, and ischemia2,3,5 and is redistributed around the cell perimeter in infarct border zones.3,4 Junctional conductance is decreased in ischemia and ventricular hypertrophy.5-9 These changes are associated with propagation slowing and sometimes arrhythmias, although other pathological changes in diseased myocardium are also likely to play a role. Recently, genetic strategies have been used to specifically examine relationships between cardiac gap junction expression and function.10-14 Using a conditional gene-targeting strategy, we generated mice deficient in myocardial Cx43 (CKO mice) and demonstrated that such mice succumb to lethal ventricular tachyarrhythmias.10 Interestingly, despite the virtual loss of the principal connexin responsible for ventricular cell coupling, effects on conduction velocity were relatively modest, with about 50% slowing.10

In this study, we directly measured transjunctional conductance in ventricular myocyte pairs from CKO mice. We found that conductance is markedly reduced to 1% to 2% of controls. This level of conductance is predicted by theoretical studies to have a much greater conduction slowing effect15-17 than observed in vivo in this model,10 suggesting that additional mechanisms support impulse propagation when gap junctional conductance is severely reduced.18

Materials and Methods

Murine Model

Gap junctional conductance was investigated in ventricular myocyte cell pairs obtained from a gene-targeted murine model in which the Cx43 gene was conditionally inactivated exclusively in cardiomyocytes by use of the Cre/loxP system (CKO mice).10 Immunohistochemical studies and Western blot analysis have demonstrated that as many as 95% of myocytes from these CKO mice fail to express Cx43.10 For controls, we used littermates that are homozygous “floxed” but do not express Cre recombinase and express wild-type levels of Cx43.19 We also studied a heterozygous group that has a ~50% reduction in Cx43 in the heart (unpublished data, 2003).

Preparation of Ventricular Myocytes

Animals were cared for according to the guiding principles in DHEW (NIH) No. 85-23. Protocols were approved by IACUC at New York University and Columbia. CKO and control mice, 4 to 6 weeks old,10 were anesthetized with ketamine (5 to 10 mg/mouse) and heparinized (200 U/mouse). Hearts were removed and perfused (at 35°C) through an aortic cannula with calcium-free HEPES buffered solution containing (in mmol/L) NaCl 118, KCl 5.6, NaHCO3 4.4, NaH2PO4 1.74, MgCl 1.69, glucose 5.6, L-glutamine 4.3, HEPES 21, tauroine 10, MEM amino acids solution (20 mL/L), and MEM vitamin solution 10 mL/L (pH 7.2). Collagenase (CLS II, 0.23 mg or 70 U/mL, Worthington Biochemicals) and trypsin (0.05 mg/mL, Worthington Biochemicals) were added to the perfusate. After 8 to 12 minutes, ventricles were triturated in this solution with addition of 0.1 μmol/L CaCl2. Collagenase was increased to 0.46 mg/mL, trypsin to 0.08 mg/mL, and 0.5% bovine serum albumin (ICN Biochemicals) was added. Dispersed cells in the supernatant were collected and resuspended in HEPES buffered solution with...
CaCl₂ increased to 1 mmol/L and 0.5% bovine serum albumin maintained.²⁰ Paired myocytes in the resuspension were studied (2 to 8 hours after disaggregation).

Electrophysiological Experiments

Electrophysiological studies were performed on both end-to-end and side-to-side coupled myocytes.⁹ To confirm that recordings were made from cell pairs, we mechanically separated the supposed pair into component cells by moving the recording pipettes. In addition, we used halothane to verify that recorded currents were transjunctional²¹ whenever possible.

Aliquots of resuspension solution containing myocytes were placed on poly-d-lysine coated coverslips in a superfusion chamber, mounted on the stage of a Nikon inverted microscope. Transjunctional currents were recorded by a double patch clamp method as previously described.⁹ Instrumentation used for whole-cell recordings and voltage-clamp protocol generation and data acquisition are described in Yao et al.⁹ Currents were low-pass filtered at 0.5 KHz with a Bessel filter (Frequency Devices) and recorded with sampling intervals of 2.5 ms.

To determine transjunctional macroscopic conductance (Gj) between paired cells, the command voltage for cell 1 (electrode 1, V₁) and cell 2 (electrode 2, V₂) were initially held at 0 mV. V₁ was then stepped to +10 mV to establish a transjunctional voltage (Vj), whereas V₂ was held at 0 mV. Maximal transjunctional conductance occurs at this Vj (see following sections). The test pulse duration was 5 seconds with an interpulse interval of 10 to 15 seconds. Detailed methods to determine Ij (macroscopic transjunctional current) and Gj are described in Yao et al²⁰ as well as descriptions of methods used to limit errors in measurements of Gj caused by series resistance.²¹,²² In our experiments, the uncompensated series resistance was 0.8 to 3.1 MΩ as determined by the methods described in Yao et al.⁹

Bath and pipette solutions were designed to minimize currents through nonjunctional ion channels (potassium, calcium [Ca++] and Na⁺–Ca++ changer currents). Sodium current (I₈Na) and residual I₈Ca were inactivated by holding membrane voltage at 0 mV. Bath solution was Ca–²⁺ and K⁺–free and composed of (in mmol/L): NaCl 146, MgCl₂ 0.5, NiCl₂ 6, BaCl₂ 1 and CsCl 2, HEPES 5, and dextrose 5.5 (pH 7.3 adjusted with CsOH). Pipette solution contained (in mmol/L): Cs-aspartate 115, TEA-Cl 20, EGTA 10, HEPES 10, ATP (Mg salt) 5, and GTP 0.5 (pH adjusted to 7.3 with CsOH).

Voltage-dependent properties of gap junctional conductance²⁵ in CKO and control pairs were compared by applying V₁ in 10-mV steps to ±100 mV. For control pairs, the low conductance (<10 nS) minimized differences between V₁ and corrected Vj to less than 10% at maximum V₁ of ±100 mV. For control pairs, transjunctional conductances were too large to permit adequate voltage control over the ±100-mV range of V₁. Therefore, conductance was reduced to <50 nS by decreasing pH in the patch pipette to 6.8. Steady-state conductance (Gmin) was measured at the end of each voltage step and normalized to instantaneous conductance (Gin) at the beginning of the voltage step. The Gmin/Vj relationship was described using the two-way Boltzmann function: Gj = (Gmax − Gmin)/{1 + exp[(AVj − Vj0)/Gmax] + Gmin}, where Gmax is maximum conductance, Gmin is sustained conductance at the end of voltage steps (also called voltage-insensitive residual conductance), Vj is transjunctional voltage halfway between Gmax and Gmin (Vj at which Gj = (Gmax − Gmin)/2), and A is a constant that defines the voltage sensitivity (see Table).

Excel (Microsoft) and SigmaStat (SPSS Science) were used for mathematical and statistical analysis of electrophysiological data. The two-sample t test was used to make comparisons of a single parameter between two independent experimental groups. When multiple comparisons were made, ANOVA with repeated measures was utilized. A P < 0.05 was considered statistically significant.

Immunofluorescence and Confocal Microscopy

Isolated myocytes from batches used for electrophysiological studies were methanol-fixed, which also permeabilizes the cells,²⁴ and stored at 4°C in phosphate-buffered saline (PBS). Cells were applied onto Superfrost/Plus microscope slides (Fisher Scientific) with a Cytospin 2 centrifuge (Thermo Shandon). Cells were washed with PBS and blocked in PBS with 5% normal goat serum, 0.1% bovine serum albumin (BSA), and 0.1% NaN₃, at 37°C for 30 minutes. Cells were incubated with appropriate primary antibodies in PBS with 1% BSA and 0.1% NaN₃ for 2 hours at 37°C. After washing in PBS, cells were incubated with conjugated secondary antibodies in PBS with 0.1% BSA and 0.1% NaN₃ at 37°C for 1 hour. Cells were washed again in PBS, mounted with Vectashield mounting medium (Vector Laboratories) and examined with an Axioskop 2 plus fluorescence microscope (Carl Zeiss Microscopy). Total number of fluorescent-stained and nonstained cells in each sample was determined by hand-count and expressed as mean percentage of fluorescent cells per preparation ± SD. Representative cells were imaged with a TCS-SP confocal laser scanning microscope (Leica).

Primary antibodies included a custom-manufactured anti-Cx43 polyclonal antibody,¹⁰,²³ anti-Cx40 (Alpha Diagnostic Intl), and anti-Cx45 polyclonal antibodies (Chemicon International). FITC-conjugated goat anti-rabbit (Jackson ImmunoResearch Laboratories) was used as the secondary antibody. Frozen sections from adult mouse atria and embryonic day 10.5 mouse heart were used as positive controls for anti-Cx40 and anti-Cx43 staining, respectively.

Results

Cx43 Immunoreactivity

Under phase-contrast microscopy, CKO and control myocytes were indistinguishable (Figure 1, right panels). Immunofluorescent staining in myocytes from control revealed typical punctate sarcosomal patterns of Cx43 expression. In single myocytes, Cx43 staining was abundant along lengths of cells (Figure 1A, matched phase contrast in Figure 1B). In cell pairs from controls, Cx43 immunoreactivity was concentrated along the border between adjoining myocytes (arrows, Figure 1C, matched phase contrast in Figure 1D). In contrast, Cx43 staining was absent in the majority of CKO myocytes, whether single (Figure 1E, matched phase contrast in Figure 1F) or paired (Figure 1G, matched phase contrast in Figure 1H).

Summary data in the bottom panel of Figure 1 show that 98.3 ± 0.2% of control single cells (mean percentage ± SD from 2 control hearts; n = 361 total cells) stained positively for Cx43, whereas only 9.3 ± 7.6% of CKO single cells stained positively for Cx43 (mean percentage ± SD from 7 CKO hearts; n = 930 total cells; P < 0.05 versus controls). In the small number of CKO cells that stained positively for Cx43, staining was similar in intensity and distribution to control cells. Cx43 was not detected in any control (n = 106)
or CKO (n=630) myocytes nor was Cx45 (control n=149; CKO n=709).

**Gap Junctional Conductance**

Figure 2A shows transjunctional current traces ($I_j$) recorded from cell 2 in end-to-end (left panel) and side-to-side (right panel) coupled control pairs. Figure 2C shows microscopic images, end-to-end at the left and side-to-side at the right. Currents generated by voltage steps of +10 mV (outward current) and −10 mV (inward current) applied to cell 1 displayed a symmetrical pattern (Figure 2A). In this example, $I_j$ of the end-to-end pair was 3.51 nA and $G_j$ was 390 nS, whereas $I_j$ of the side-to-side pair was 3.47 nA and $G_j$ was 387 nS. Amplitudes of $I_j$ recorded from CKO myocyte pairs (Figure 2B) (microscopic images in Figure 2D of end to end [left] and side to side [right]) were much smaller compared with controls. In this example, $I_j$ of the end-to-end pair (left panel) was 0.12 nA and $G_j$ was 12.2 nS, whereas $I_j$ of the side-to-side pair (right panel) was 0.07 nA and $G_j$ was 7.1 nS.

Figure 3 summarizes gap junctional conductance at a transjunctional voltage ($V_j$) of +10 mV for all pairs studied in all groups. Conductance at this voltage is close to maximal (see following section). In control, averaged conductance was $588\pm104$ nS (range 226 to 981 nS) (n=6) for side-to-side coupled pairs and $558\pm92$ nS (range 262 to 937 nS) (n=7)
for end-to-end coupled pairs (not significantly different; 
\( P > 0.05 \) versus side-to-side coupled pairs). In heterozygous 
Cx43 pairs, conductance was 258±62 nS (range 24 to 397 
nS) for side-to-side (\( n=4 \), \( P < 0.05 \) versus control) and 
244±67 nS (range 36 to 460 nS) for end-to-end pairs (\( n=4 \), 
\( P < 0.05 \) versus control). There was no significant difference 
between side-to-side and end-to-end coupled pairs (\( P > 0.05 \)). 
In both of these groups, all cell pairs had measurable 
conductance. In 79% (15 of 19) of CKO pairs, measurable 
transjunctional conductance occurred, whereas in the remaining 
21% (4 of 19), no transjunctional current flow occurred 
despite demonstration of physical coupling of cells (see 
Materials and Methods). Transjunctional conductance for the 
entire group was 4\( \pm \)2 nS (range 0 to 19 nS) for side-to-side 
coupled pairs (\( n=9 \), \( P < 0.05 \) versus side-to-side and end-to-
end control and heterozygous groups) and 11±2 nS (range 2 
to 23 nS) for end-to-end coupled pairs (\( n=10 \), \( P < 0.05 \) versus 
end-to-end and side-to-side coupled control and heterozygous 
groups). Mean conductance from only cell pairs that had 
measurable conductance was 6\( \pm \)2 nS for side-to-side and 13\( \pm \)2 
nS for end-to-end coupled pairs (still significantly 
reduced versus control and heterozygous pairs).

### Characteristics of Residual Conductance in 
CKO Myocytes

Although immunostaining showed that 90% of myocytes 
from CKO mice were Cx43 negative, electrophysiological 
studies demonstrated that 79% of CKO pairs had measurable 
transjunctional conductance. Therefore, a residual transjunc-
tional conductance occurs in cells in which Cx43 is geneti-
cally ablated. To explore the nature of this residual conduc-
tance, we examined both its sensitivity to halothane, a 
relatively specific gap junction channel blocker, \(^{21}\) and its 
voltage-dependent properties.

Figure 4 shows results of an experiment with halothane 
(10 mmol/L) (residual transjunctional conductance was 10 
nS). Conductance was completely blocked within 3 minutes 
(same effect in all 4 CKO pairs studied). Thus, residual 
conductance in CKO myocytes is likely dependent on con-
nexin gap junctional channels rather than alternate methods 
of current flow between cells. \(^{18}\)

To obtain voltage dependence profiles of controls, the large 
transjunctional conductance, \( G_j \), was reduced so that adequate 
membrane voltage clamp control could be achieved. By 
Titration of intracellular pH \([\text{pHi}]\) to 6.8, from control of 7.3 
(see Materials and Methods), \( G_j \) was reduced to 8.0\( \pm \)1.3 nS 
in 8 control pairs, a level comparable to CKO pairs. Figure 
5A shows transjunctional current \( (I_j) \) traces in a control pair at 
low pH\(_i\) during an experiment in which transjunctional 
voltages \( (V_j) \) between \( \pm 100 \) mV were applied in 10-mV steps. 
Instantaneous currents \( (I_{j,in}) \), current amplitude at the begin-
ing of the \( V_j \) steps) were linearly related to \( V_j \) over all 
applied voltages (Figure 5C, unfilled circles). Steady-state 
current \( (I_{j,ss}) \), current amplitude at the end of \( V_j \) steps) 
displayed a linear relation to \( V_j \) only between 30 and 30 
mV (Figure 5C, solid circles). Current rectification occurred 
at \( V_j > 40 \) mV and \( < -40 \) mV where \( I_j \) declines to a steady 
state value (Figure 5A and solid circles in C).
Figure 5B shows $I_j$ of a CKO pair (pH 7.3) in response to $V_j$ in 10-mV steps, between $-100$ and $+100$ mV. As in controls, $I_{j,in}$ is linearly related to voltage (Figure 5D, unfilled circles), whereas $I_{j,ss}$ shows rectification (Figure 5B and solid circles in Figure 5D). However, the currents in CKO pairs show stronger rectification than controls.

The steady state conductance ($G_{j,ss}$)–$V_j$ relationship of control pairs is displayed as a symmetrical bell-shaped curve in Figure 6A (unfilled circles) such that normalized $G_{j,ss}$ values of adult myocytes.9,22 There are no previously published gap junctional conductance data on adult mouse ventricular myocytes.9,22 Values for our controls are comparable to those previously reported for adult ventricular myocyte pairs from canine ventricles. The $G_{j,ss}$ of control mouse pairs at pH 6.8. Therefore, at lower pHi properties of voltage dependence of control gap junction channels can still be identified as Cx43 dependent, and it appears that they were not altered by lowering the pH.

Steady-state $G_{j,ss}$–$V_j$ relationship of residual $G_j$ in CKO pairs (Figure 6A, solid circles) is shifted to lower voltages compared with controls. $V_j$ in CKO pairs was $45.2 \pm 1.5$ mV compared with $+65.2 \pm 1.7$ mV in control pairs ($P<0.05$) in positive voltage polarity, and $-45.0 \pm 1.8$ mV compared with $-61.1 \pm 1.4$ mV in control pairs ($P<0.05$) in the negative polarity (Table). Therefore, residual conductance in CKO pairs has stronger voltage dependence. Other Boltzmann parameters for CKO myocytes in the Table were not significantly different from control, except for $G_{j,ss}$.

In control pairs, the time course of current decay at $V_j>70$ mV and $<-70$ mV was best described by a double exponential function. Fast and slow time constants ($r_f$ [circles] and $r_s$ [squares] in Figure 6B) became smaller as $V_j$ increased; $r_f$ and $r_s$ at a $V_j$ of $-80$ mV were $0.50 \pm 0.10$ and $1.76 \pm 0.35$ seconds (n=9), respectively. The $r_f$ and $r_s$ at a $V_j$ of $-100$ mV were $0.27 \pm 0.04$ seconds ($P<0.05$ versus $r_f$ at $-80$ mV) and $1.49 \pm 0.32$ seconds ($P<0.05$ versus $r_s$ at $-80$ mV), respectively. In CKO pairs, the fast component of current decay was significantly accelerated. $r_f$ at $-80$ mV and $-100$ mV were $0.23 \pm 0.03$ seconds (n=8, $P<0.05$ versus control) and $0.08 \pm 0.01$ seconds (n=8, $P<0.05$ versus control), respectively. The $r_f$ were reduced at all voltages tested, although the reduction did not reach statistical significance.

**Discussion**

We evaluated cell coupling in ventricles of control and Cx43-deficient (CKO) mice by determining gap junctional conductance, $G_j$, between myocyte pairs using a dual patch clamp technique, minimizing errors inherent in investigations of adult myocytes.9,22 There are no previously published gap junctional conductance data on adult mouse ventricular myocytes. Values for our controls are comparable to those previously reported for adult ventricular myocyte pairs from canine ventricular myocytes. There are no previously published gap junctional conductance data on adult mouse ventricular myocytes. Values for our controls are comparable to those previously reported for adult ventricular myocyte pairs from canine ventricular myocytes.
immunofluorescence. If we compare the percentage of Cx43, with the majority of ventricles devoid of areas of ventricular tissues in CKO mice staining positive agreement with our previous study in which we found rare physiological study showed that 90% of myocytes had no detectable Cx43. In 10% of myocytes staining positively for Cx43, results show that the majority of myocyte pairs from the CKO mice have markedly reduced, albeit measurable levels of intercellular coupling.

Figure 6. A, Normalized steady-state conductance ($G_{j,ss}$)-transjunctional voltage ($V_j$) relationships. Unfilled and solid circles represent mean values of normalized $G_{j,ss}$ from control (n=9) and CKO (n=10), respectively. Symbols and vertical bars denote mean±SE. Smooth curves superimposed on data points are calculated based on two-way Boltzmann function with best-fit parameters (see Table for summary of parameters). *$P<0.05$ of CKO data points vs control. B, Voltage dependence of time constants of current decay. Time courses of $I_j$ decay fit with double-exponential function (inset). Circles and squares stand for means of fast ($s_f$) and slow ($s_s$) time constants, respectively. Vertical bars stand for SE. Numbers of observations are in parentheses. *$P<0.05$ vs control.

Our results show that the majority of myocyte pairs from the CKO mice have markedly reduced, albeit measurable levels of coupling. Immunofluorescent study of CKO myocytes from the same aliquots from which cell pairs were obtained for electrophysiological study showed that 90% of myocytes had no detectable Cx43. In 10% of myocytes staining positively for Cx43, its levels appeared similar to control. These results are in agreement with our previous study in which we found rare areas of ventricular tissues in CKO mice staining positive for Cx43, with the majority of ventricles devoid of immunofluorescence. If we compare the percentage of myocytes from CKO hearts devoid of Cx43 (90%) with data from the electrophysiological study showing that 79% of myocyte pairs maintained some degree of electrical coupling, the conclusion can be made that electrical coupling occurred in cell pairs that did not have detectable levels of Cx43 by immunofluorescence. Moreover, given the genetic strategy used to produce these mice in which Cx43 expression in myocytes is decreased by either ~50% (one allele knocked-out) or 100% (both alleles knocked out), we can assume that the residual conductance in CKO cells is not Cx43-dependent. However, halothane sensitivity suggests coupling through gap junctions formed by connexin proteins.

Biophysical properties of channels formed from individual connexins are unique. Therefore, we compared voltage dependence of channels in CKO pairs with that in control, to determine if the electrophysiological signature could help identify the molecular basis of the residual conductance. To characterize voltage dependence of control myocytes, it was necessary to reduce conductance to levels at which adequate voltage control could be maintained with clamp pulses up to ±100 mV, by lowering pH in the pipette to 6.8. In preliminary studies in canine ventricular myocyte pairs, we found that decreasing pH to 6.8 did not change voltage dependent properties of Cx43 channels. This assumption is also verified by results of studies reported here, that show typical Cx43 voltage dependent properties of gap junctions in control at low pH, when compared with our previous study on canine ventricular cell pairs and expressed Cx43 gap junctional channels.

Gap junctional conductance in CKO myocytes showed a steeper voltage dependence than Cx43 gap junctions in control myocytes. Among the three major cardiac connexins, the steepest voltage dependence arises from channels composed of Cx45. Other investigators have reported expression of low levels of Cx45 in adult murine ventricle. As in our recent report, we failed to detect Cx45 in either control or CKO cell-pairs, although this may simply reflect differences in technique. Assuming a single-channel conductance value of ~32 pS for channels comprised of Cx45, the macroscopic conductance of 11 nS in CKO cell-pairs can be accounted for by as few as 350 channels. This level of expression may simply be below the threshold of detection of the Cx45 antisera, at least in our hands. Alternatively, the residual coupling may represent expression of a connexin isoform other than Cx45.

Conditional inactivation of the Cx43 gene exclusively in cardiomyocytes in this genetically engineered murine model is characterized by normal heart development and contractile function and avoidance of perinatal lethality observed in the germline knockout. However, the incidence of sudden death from ventricular tachyarrhythmias increases between 4 and 6 weeks after birth and ultimately occurs in all CKO mice. Conduction velocities from epicardial optical mapping during this time period were shown to be decreased approximately 50% in both longitudinal and transverse directions. Subsequent studies have identified hearts with conduction velocities as slow as ~20% of normal values (G.E. Morley, D.E. Gutstein, G.I. Fishman, unpublished data, 2003). However, a greater decrease in conduction velocity might be expected based...
on our conductance data. In a computer model, Shaw and Rudy\textsuperscript{15} found that a decrease in gap junction conductance to 6pS associated with a conduction velocity of less than 1 cm/sec. Rohr et al\textsuperscript{16} in myocyte strands in culture exposed to the uncoupling agent palmitoleic acid showed conduction velocity to be reduced to less than 1 cm/sec.

There are several possible explanations for the discrepancy between gap junctional conductance from our experiments on cell pairs and conduction velocity measured in whole hearts. (1) The discrepancy may be artificial. Although control myocyte pairs were electrically well coupled after enzymatic disaggregation, coupling of CKO myocytes may be more vulnerable to these procedures owing to absence of Cx43. Previous studies have also shown that gap junctional connexins can be internalized when cells are isolated,\textsuperscript{43–45} which could lead to a reduction in conductance that we measured in the isolated cell pairs that does not reflect the actual conductance in vivo. (2) Another explanation for the discrepancy is provided in a computer model of Kucera et al\textsuperscript{17} showing that conduction is influenced by leak currents and potentials that result from localization of sodium channels to clefts. Under conditions of substantially reduced gap junctional conductance, interactions between $I_{\text{leak}}$ and cleft potentials resulted in enhancement of conduction when there were narrow intercellular clefts.\textsuperscript{17} Sperelakis has previously proposed that electrical transmission can occur between myocytes not connected by low resistance pathways by virtue of the electric field that develops in narrow junctional clefts.\textsuperscript{18,46} (3) A recent study by Thomas et al\textsuperscript{47} has shown an increased upstroke velocity in cultured myocytes with reduced Cx43, possibly resulting from upregulation of sodium current, that may play a role in maintaining conduction. (4) The smaller diameter of murine myocytes renders total axial resistance less dependent on gap junctional resistance and more on myoplasmic resistance.\textsuperscript{47} In the absence of direct experimental data, it is not possible to state with any certainty how each of the four possibilities contribute to our results.

In summary, electrical coupling between myocytes from CKO hearts is dramatically reduced. Residual conductance is likely due to low levels of alternative connexin isoforms with greater voltage-dependence, such as connexin45. The observation that conductive velocity is relatively preserved in vivo in the presence of low levels of intercellular coupling that we measured in dissociated cell pairs is intriguing and additional experimentation will be necessary to define the mechanism(s) underlying our findings. It is likely, however, the, CKO mice will continue to provide additional insights into molecular determinants of conduction associated with gap junction remodeling and their relationship to arrhythmogenesis.

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