Focal gap junction uncoupling and spontaneous ventricular ectopy

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Gutstein, David E., Stephan B. Danik, Steve Lewitton, David France, Fangyu Liu, Franklin L. Chen, Jie Zhang, Newsha Ghodsi, Gregory E. Morley, and Glenn I. Fishman. Focal gap junction uncoupling and spontaneous ventricular ectopy. Am J Physiol Heart Circ Physiol 289: H1091–H1098, 2005.—Genetic studies in the mouse have demonstrated that conditional cardiac-restricted loss of connexin43 (Cx43), the major ventricular gap junction protein, is highly arrhythmogenic. However, whether more focal gap junction remodeling, as is commonly seen in acquired cardiomyopathies, influences the propensity for arrhythmogenesis is not known. We examined electrophysiological properties and the frequency of spontaneous and inducible arrhythmias in genetically engineered chimeric mice derived from injection of Cx43-deficient embryonic stem cells into wild-type recipient blastocysts. Chimeric mice had numerous well-circumscribed microscopic Cx43-negative foci in their hearts, comprising ∼15% of the total surface area as determined by immunohistochemical analysis. Systolic function in the chimeric mice was significantly depressed as measured echocardiographically (19.0% decline in fractional shortening compared with controls, P < 0.05) and by invasive hemodynamics (17.6% reduction in change of pressure over time, P < 0.01). Chimeras had significantly more spontaneous and inducible arrhythmias than controls (P < 0.01), including frequent runs of nonsustained ventricular tachycardia in some of the chimeric mice. However, in contrast to mice with conditional cardiac-restricted loss of Cx43 in the heart, no sustained ventricular arrhythmias were observed. We conclude that focal areas of uncoupling in the myocardium increase the likelihood of arrhythmic triggers, but more widespread uncoupling is required to support sustained arrhythmias.

Sudden arrhythmic death is a common cause of mortality in many forms of heart disease (4, 23, 36, 40). Abnormal intercellular coupling in the heart, resulting from defects in expression of connexin43 (Cx43), the major constituent of cardiac gap junctions, plays a key role in the initiation and maintenance of sustained arrhythmias (7, 15, 25, 28, 38). Abnormal Cx43 expression is seen in many common forms of acquired heart disease, although gap junction remodeling in these conditions can be focal in nature and preferentially affect ischemic or injured myocardial segments (9, 19, 22, 35). Nonetheless, these relatively restricted regions of gap junction remodeling may play a key mechanistic role in arrhythmogenesis, as demonstrated in the canine infarct model, where alterations in Cx43 expression in the infarct border zone correlate with the location of reentrant circuits (32).

We have previously established that cardiac-restricted conditional knockout (CKO) of Cx43, in which Cx43 expression is inactivated in the great majority of myocytes, results in a highly arrhythmogenic substrate (7, 13, 15). To investigate the role of focal uncoupling in cardiac function, we have also developed a murine model of heterogeneous gap junction expression by generating chimeric animals from the introduction of Cx43-deficient embryonic stem (ES) cells into wild-type recipient blastocysts (16). Gap junction expression in these hearts is highly abnormal, with well-circumscribed foci of Cx43-deficient myocytes interspersed throughout an otherwise well-coupled myocardial syncitium. Interestingly, these mice had depressed systolic function in the absence of ventricular dilatation or hypertrophy, conceivably a consequence of asynchronous myocardial excitation and contraction (16). Interestingly, during the course of evaluation of impulse propagation in the chimeras, two of seven isolated-perfused hearts developed spontaneous ventricular tachycardia, as opposed to zero of six control hearts, suggesting increased arrhythmic susceptibility. Accordingly, in the present study, we have performed a detailed analysis of the in vivo electrophysiological phenotype in Cx43 chimeric mice. We documented a significant increase in spontaneous ventricular ectopy, including some mice with frequent runs of nonsustained ventricular tachycardia, a finding never seen in controls. However, in marked contrast to CKO mice, which have widespread loss of Cx43 in the myocardium (15), these chimeric mice did not develop sustained ventricular tachycardiac, nor were such arrhythmias inducible by programmed electrical stimulation (PES). These data highlight the importance of uncoupled foci as arrhythmic triggers and suggest that additional pathology such as more widespread uncoupling or myocardial injury is required for the maintenance of sustained arrhythmias.

MATERIALS AND METHODS

Generation of chimeric mice and genotyping. Studies involving the Cx43 chimeric mice were approved by the Institutional Animal Care and Use Committees of the New York University School of Medicine, the New York Harbor Veterans Administration Medical Center, and the Mount Sinai School of Medicine (New York, NY) and performed in compliance with National Institutes of Health guidelines. The generation of homozygous Cx43-null ES cells and their injection into wild-type blastocysts was performed as described previously (16). To compare the incidence of spontaneous ectopy in unanesthetized chimeric and control mice, a pilot series of four chimeric mice (see chimeras 1–4 in Table 3) were generated by injection of 129/Sv ES cells into C57BL/6 host blastocysts, followed by reimplantation into pseudopregnant foster mothers. Controls for this pilot series consisted...
of 129/Sv X C57BL/6 F1 age- and sex-matched mice (see controls 1 and 2 in Table 3). Data from the pilot series were used only for the comparison of spontaneous ectopic events in telemetry-monitored unanesthetized mice. In addition to the pilot series, additional chimeric mice were generated in which 129/Sv ES cells were injected into 129/Sv host blastocysts. These chimeras were used to compare the incidence of spontaneous ventricular ectopy, as well as for all other comparisons. Purebred age- and sex-matched 129/Sv mice were used as controls for these subsequent series of chimeras.

Genomic DNA from chimeric offspring was prepared as described previously (24). Southern blotting was performed by using a 700-bp probe, adjacent to the 3’ end of the Cx43 open reading frame, yielding the expected 6.5-kb wild-type band and the 4.3- and 3.4-kb knockout bands (16).

Histology and immunofluorescence. For the determination of Cx43 expression patterns in chimeric hearts, immunostaining was performed in frozen sections that were blocked and then incubated with a polyclonal anti-Cx43 primary antibody (14). After being washed in PBS, sections were incubated with a FITC-conjugated goat anti-rabbit antibody (Jackson ImmunoResearch, West Grove, PA) and mounted with Vectashield mounting medium (Vector, Burlingame, CA).

Stained sections were visualized on an Axioskop 2 Plus fluorescence microscope, and images were collected by using an AxioCam camera with AxioVision 2.05 software (Carl Zeiss, Munchen-Hallbergmoos, Germany). IPLab 3.5.5 software (Scanalytic, Fairfax, VA) was used to compare the mean fluorescence of Cx43-positive regions after subtracting the background fluorescent intensity from each sample (14).

To determine the typical sizes of Cx43-deficient regions, 20 short-axis ventricular sections, separated from each other by 40 μm (for a total survey area of 920 μm in each of 2 Cx43 chimeric hearts), were immunostained for Cx43. The maximum distance across discrete areas devoid of Cx43 staining and the minimal distances between these regions were measured by using AxioVision software.

Immunoblotting and densitometry. Evaluation of Cx43 protein levels was performed in total ventricular lysates from six controls and six chimeras that were prepared by Dounce homogenization in the presence of Complete protease inhibitor cocktail (Roche, Mannheim, Germany). Immunoblotting was performed with equal concentrations of Cx43 open reading frame, yielding a 200-m platinum monopolar-stimulating electrode (Frederick Haer, Bowdoinham, ME). Immunoblotting and densitometry were performed with a model 2352 Programmable Stimulator (Medtronic, Minneapolis, MN). Signals were amplified by using a Honeywell ECG amplifier (Honeywell, Morristown, NJ), converted from analog to digital with the ACQ-16 Acquisition Interface and recorded with Ponemah Physiology Platform software (Gould, Valley View, OH). Output was set at twice the stimulating threshold. To determine the ventricular effective refractory period (VERP), a train of eight stimuli at cycle lengths of 120, 100, and 80 ms was delivered with single extrastimuli introduced at progressively shorter intervals. The pacing protocol was repeated in each animal at a second pacing site, and the VERP results were averaged. Double extrastimuli at each cycle length and each pacing site were added to assess inducibility of ventricular arrhythmias. After the study, the electrode was removed and the animals were sutured closed and allowed to recover.

Echocardiography. Echocardiography was performed in the same cohort of Cx43 chimeric mice at 10 mo of age, according to a previously described protocol (15, 16). Briefly, mice were anesthetized with tribromoethanol (Avertin, 20 mg/kg ip, 1.2 vol% solution) and imaged by using an Acuson Sequoia echocardiography machine and a 15-MHz linear probe. Measurements were performed online by using customized software generously provided by Acuson.

Hemodynamics. Invasive hemodynamic assessment was performed from analog to digital with the ACQ-16 Acquisition Interface and recorded with Ponemah Physiology Platform as described above. After the procedure, mice were given a lethal dose of 500 mg/kg Avertin ip, and their hearts were removed.

Statistics. Data are expressed as means ± SE. Assessment of spontaneous ectopy in controls and chimeras was performed by using the method of Brunner et al. (3), by comparing with a £2 analysis the number of mice in each group with greater than 5 episodes of spontaneous ventricular ectopy per 24 h of monitoring (Georgetown Linguistics Web Chi Square Calculator). Because of the nonnormal distribution of values for ventricular premature beats and total arrhythmic events, these values were log transformed and then compared with a two-tailed t-test (Microsoft Excel). Percentage of chimerism between epi-, mid-, and endocardium was compared with ANOVA by using StatView (SAS Institute, Cary, NC). All other comparisons between groups were performed with a two-tailed t-test (Microsoft Excel). P < 0.05 was considered statistically significant.

RESULTS

Chimerism is detectable on Southern blot analysis. To generate Cx43 chimeric mice, homozygous Cx43-null ES cells derived from 129/Sv mice were injected into wild-type 129/Sv recipient blastocysts and implanted into pseudopregnant females in two separate surgeries. Of the resulting 14 pups, 7
pups had evidence of chimerism determined by Southern blot analysis (Fig. 1A). The chimeric pups were grossly indistinguishable from the nonchimeric littersmates. There were no spontaneous deaths in this cohort of chimeric mice over the 10-mo observation period.

Cx43 chimerism causes ventricular dysfunction without hypertrophy or dilatation. We utilized echocardiography to noninvasively assess left ventricular function, chamber size, and wall thicknesses. In our previous study of chimeric Cx43 knockout mice (16), we injected ES cells from a C57BL/6 background into 129/Sv host blastocysts. Because the two strains are different sizes in adulthood, we were unable to directly compare chamber sizes. A direct comparison of ventricular size was possible in this series of chimeras because both ES cells and recipient blastocysts were derived from the 129/Sv strain, thus removing strain differences as a complicating factor.

Global ventricular function, as measured by fractional shortening, was significantly reduced in the chimeric mice compared with age- and strain-matched controls (Table 1). Fractional shortening in the chimeras was 33.2 ± 2.08% compared with 41.0 ± 1.27% in the controls (P < 0.05). This difference in fractional shortening represented a 19% decline in global ventricular function in the chimeras compared with the con-
controls. Similarly, fractional shortening was significantly reduced in our previous series of Cx43 chimeras (16). There were no significant differences in end-diastolic left ventricular dimensions or wall thicknesses, indicating that despite a significant decline in global function, there was no echocardiographic evidence of ventricular dilatation or hypertrophy in the chimeras.

Isolated systolic dysfunction in chimeric mice. Immediately before death, chimeric and control mice were studied with invasive hemodynamic monitoring (Table 2). Consistent with the echocardiographic analysis, overall contractility in the chimeric mice was significantly reduced. Contractility, as measured by maximum change of pressure over time ($dP/dt_{\text{max}}$), was 7,147 ± 248 mmHg/s in the chimeras compared with 8,673 ± 283 mmHg/s in the controls ($P < 0.01$), representing a 17.6% decline in the chimeras. Interestingly, indexes of diastolic function, including minimum change of pressure over time ($dP/dt_{\text{min}}$) and Tau, were not significantly changed in the chimeric mice compared with controls. Although both systemic and left ventricular systolic pressures were decreased in the chimeras, there was no difference in diastolic pressures or heart rate.

Augmented Cx43 expression in nonchimeric areas of chimeric hearts. To assess ventricular expression of Cx43 in the chimeric hearts on an ultrastructural level, immunofluorescent staining was performed (Fig. 1, B and C). As expected, staining for Cx43 in the chimeric hearts showed focal loss of gap junctions throughout the heart, with randomly distributed areas lacking Cx43 staining. Areas devoid of Cx43 (Cx43 negative) were well circumscribed and clearly demarcated from the adjacent Cx43 expressing (Cx43 positive) myocardium (arrows, Fig. 1C). Cx43-negative regions constituted 16.2 ± 5.7% of epicardial sections surveyed in the chimeric hearts, 15.0 ± 3.2% of midmyocardial sections and 12.4 ± 2.2% of endomyocardial sections [6 randomly chosen images from each myocardial level were assayed for each of 6 chimeric hearts; $P = \text{not significant (NS)}$ for comparisons between myocardial layers].

Two of the chimeric hearts were surveyed to determine the typical sizes of Cx43-deficient regions. In one of the chimeric hearts, there was an average of 5.5 ± 0.32 Cx43-deficient regions per section (range 4–7) or one Cx43-deficient region per 2.3 mm$^2$ of chimeric myocardium. The maximum dimension of these regions averaged 227.0 ± 11.2 μm (range 73.5–446.4 μm), and they were 330.9 ± 39.7 μm (range 15.6–971.3 μm) from the nearest Cx43-deficient region. In the second chimeric heart surveyed, there was an average of 4.5 ± 0.25 Cx43-deficient regions per section (range 3–6; one Cx43-deficient region per 5.6 mm$^2$ of chimeric myocardium); the maximum dimension averaged 494.8 ± 62.4 μm (range 139.8–1422.2 μm), and they were separated from the nearest Cx43-null region by 161.0 ± 25.2 μm (range 10.0–601.3 μm). Thus Cx43-deficient zones constitute relatively large and well-demarcated areas of the chimeric ventricle, likely accounting for discrete areas of conduction delay and disruption of the activation wave front.

Interestingly, compared with wild-type hearts ($n = 6$), the abundance of Cx43 in the Cx43-positive regions of the chimeric hearts ($n = 6$) was significantly greater (126 ± 5.9% of control, $P < 0.05$; Fig. 1D). Consistent with these immunofluorescent findings suggesting a compensatory upregulation in Cx43-positive regions, we found that despite substantial areas devoid of Cx43 in the chimeric hearts, there was no significant difference in overall Cx43 protein content by immunoblotting compared with wild-type hearts (chimeric ventricular Cx43 protein content was 114 ± 11% of control, $P = \text{NS}$; Fig. 1, E and F).

Spontaneous ventricular ectopy in chimeric mice. To analyze the occurrence of spontaneous ventricular arrhythmias in the chimeric mice, we surgically implanted telemeters and recorded electrocardiographic activity. Recordings from unchimeric mice ($n = 10$) were obtained and compared with age- and sex-matched controls ($n = 8$). Ventricular ectopy was detected by using custom-made software and was confirmed by manual review. With the use of the criteria of Brunner et al. (3) (>5 ventricular arrhythmic events per 24 h), all 10 of the chimeric mice and only 3 of the 8 controls had evidence of frequent ventricular ectopy ($P < 0.01$; Table 3). Chimeric mice had significantly more arrhythmic events than controls irrespective of the time of day ($P < 0.01$; Fig. 2). The incidence of premature ventricular complexes (PVCs) was significantly increased in the chimeras (230.1 ± 95.6 per mouse per 24 h) compared with controls (10.2 ± 6.0; $P < 0.05$). Interestingly, because PVC morphology was limited to one or two types per control mouse, there were significantly more PVC morphologies in each of the chimeric mice (0.8 ± 0.4 PVC morphologies per control mice, 6.0 ± 2.0 per chimera, $P < 0.05$; Fig. 3). This suggests that whereas control mice may have isolated foci generating premature ectopic complexes,
chimeric mice have many more such foci. No relationship was
detected between the frequency of ventricular ectopy and
reduced ventricular function by echocardiography or invasive
hemodynamics, suggesting that the cardiomyopathy noted in
the chimeras is not tachycardia induced.

Complex ventricular ectopy was limited to the chimeric
mice. There were frequent runs of nonsustained ventricular
tachycardia in 4 of 10 chimeric mice (Fig. 4). Runs of ventricu-
lar tachycardia in the chimeras ranged from 3 to 141 beats
(maximum of 5.6 s) at cycle lengths of up to 33 ms. In
comparison, there was no ventricular tachycardia in any of the
controls (P < 0.05 compared with chimeric mice).

Electrophysiological indexes are unchanged in chimeric
mice. We hypothesized that focal areas of conduction delay
within the myocardium caused by heterogeneous loss of gap
junctions would provide a substrate for reentrant ventricular
arrhythmias. To test this hypothesis, we subjected the chimeric
mice and matched controls to electrophysiological study with
PES. We have previously described an experimental protocol
for determining the phenotype. In the CKO mice, myocytes devoid
of Cx43, as opposed to generalized uncoupling of cardiac myocytes, may increase the propensity for the devel-
operator of ventricular arrhythmias.

The behavior of the Cx43 chimeras contrasts with that
observed in mice with widespread loss of Cx43 in the heart (i.e.,
CKO mice), which develop sudden arrhythmic death by 2 mo
of age and are easily inducible into sustained ventricular
arrhythmias (13, 15). Taken together, these studies indicate
that the spatial pattern of uncoupling must play a key role in
determining the phenotype. In the CKO mice, myocytes devoid
of Cx43 expression are dispersed randomly in a microscopic

Table 3. Spontaneous ectopy in unanesthetized telemetry
monitoring of Cx43 KO chimeric and wild-type control mice

<table>
<thead>
<tr>
<th></th>
<th>PVCs, 24 h</th>
<th>Couplets, 24 h</th>
<th>Runs of VT, 24 h</th>
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<tr>
<td>Controls</td>
<td>107</td>
<td>0</td>
<td>0</td>
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<tr>
<td></td>
<td>207</td>
<td>0</td>
<td>0</td>
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<td></td>
<td>307</td>
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<td>1007</td>
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Values are means ± SE. PVC, premature ventricular complex; VT, ventric-
ular tachycardia.

DISCUSSION

Abnormal gap junction expression in the heart has been
described in both animal models and clinical pathological
studies of heart disease. For example, gap junction remodeling
has been observed in both ischemic heart disease and end-stage
dilated cardiomyopathy (9, 19, 22, 35). However, in these
clinical entities, abnormal expression of Cx43 may be re-
stricted to areas immediately adjacent to the injured myocar-
dium, whereas unaffected areas of the heart maintain relatively
normal Cx43 expression (19). Despite the often limited extent
of gap junction remodeling, isolated areas of abnormal Cx43
expression have been shown to correlate with the location of
reentrant circuits in the canine epicardial infarct border zone
(32). In this study, we investigated whether isolated areas
devoid of Cx43, as opposed to generalized uncoupling of cardiac myocytes, may increase the propensity for the develop-
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arrhythmias (13, 15). Taken together, these studies indicate
that the spatial pattern of uncoupling must play a key role in
determining the phenotype. In the CKO mice, myocytes devoid
of Cx43 expression are dispersed randomly in a microscopic

No disruption of the diaphragm, respiratory distress, or
procedural mortality was noted. Baseline electrocardiographic
indexes (Table 4) were not significantly different between
chimeric mice and controls. Despite focal areas of conduction
delay on optical mapping of isolated chimeric hearts (16), the
QRS duration in chimeric mice was not significantly pro-
longed. There were no significant differences in VERP be-
tween the chimeric and control mice at any of the cycle lengths

Total arrhythmic events in chimeric Cx43 KO (●) and control mice (○) are
arranged by time of day from 1 (1 AM) to 24 (12 AM). Chimeric Cx43 KO
mice have significantly more arrhythmic events than controls (P < 0.01).

![Fig. 2. Incidence of ventricular arrhythmic events during 24-h monitoring.](http://ajpheart.physiology.org/)

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heterogeneous pattern throughout the heart without discrete “knocked-out” areas. As a result, the conduction wave front measured with optical mapping remains smooth, but impulse propagation becomes progressively slower as a function of the extent of the knockout (7, 15). When the abundance of Cx43 falls by at least 60% compared with controls, the likelihood of inducing sustained ventricular arrhythmias is substantial (7).

In contrast, chimeric Cx43\(^{-/-}\) hearts have discrete regions lacking Cx43 expression and corresponding focal areas of conduction delay, rather than global slowing of conduction velocity (16). This focal abnormality is associated with an increased level of ectopy, but the substrate does not appear to support sustained arrhythmias. What accounts for this increase in ectopy? It is well known that diminished coupling isolates pacemaker cells from surrounding tissue to provide the appropriate source-sink relationship for successful impulse propagation from node to working myocardium (2). Primary pacemaker regions of both the sinoatrial and atrioventricular nodes have a reduced or absent expression of Cx43 (6, 10, 18) and correspondingly elevated levels of intercellular resistivity (1). It is conceivable that the discrete areas of uncoupled myocardium, such as those in the chimeric Cx43\(^{-/-}\) heart, may mimic this behavior and unmask ectopic foci. This may explain why both the absolute quantity of ectopy and the number of unique morphologies were greater.

Additionally, focal areas of conduction delay caused by chimeric Cx43-deficient clusters of myocytes may disrupt the activation wave front, leading to the formation of wave breaks. These, in turn, may generate reentrant circuits. However, given the lack of sustained arrhythmias, these reentrant circuits must be unstable and short-lived, perhaps in part because global conduction velocity is not significantly slowed (5, 30, 39). Moreover, intervening Cx43-positive regions in the chimeric hearts appear to have supranormal levels of Cx43 expression, which may increase conduction velocity in these areas (8). Theoretically, the increased conduction velocity may lengthen wavelength (the

Fig. 3. Chimeric Cx43 KO mice display many different premature ventricular complex (PVC) morphologies. In contrast to the controls, each chimeric mouse showed many different PVC morphologies. Representative examples of PVCs from different days in the same control and chimeric mice are shown, demonstrating a consistent PVC morphology in the control over 6 days of monitoring and several different PVC morphologies in the chimera over the same time span.
product of conduction velocity and effective refractory period), making sustained reentry less likely.

This study may have important implications for the emerging field of cell transplantation for end-stage heart failure. Transplantation of skeletal myoblasts into the diseased myocardium has been recognized as potentially arrhythmogenic in humans (27), despite positive effects on ventricular function in experimental models of myocardial infarction (11, 17, 27). As grafted myoblasts differentiate into myotubes, they lose expression of both gap junctions and adherens junctions but maintain the ability to contract when electrically stimulated (29, 33). Thus the increased arrhythmogenicity seen in myoblast-derived cardiomyocyte transplantation may result from a myopathic ventricle that is suddenly challenged with an acute increase in the frequency of arrhythmic triggers. Moreover, our observations raise a cautionary note for genetically engineered transplantation strategies in which the proliferative potential of poorly coupled cells is enhanced. It is conceivable that a critical threshold may be reached where foci of such cells reach sufficient size that areas of conduction delay or arrhythmic triggers as well as the substrate for reentrant behavior are more likely.

We previously demonstrated that focal areas of conduction delay are associated with systolic dysfunction, presumably a reflection of diminished synchrony of contraction. This result was confirmed in this study by using strain-matched ES cells and recipient embryos. Interestingly, human clinical trials demonstrate that restoration of synchrony with biventricular pacing can ameliorate contractile deficits (12, 20, 21, 26). These observations raise the possibility that reversal of gap junction remodeling may not only diminish arrhythmic risk but may also lead to improvements in systolic performance. Although the QRS duration was not prolonged in the chimeric mice, recent studies (31, 34) indicate that resynchronization therapy may benefit patients with asynchronous contraction independent of QRS duration.

In summary, these data in concert with our earlier studies suggest that abnormal gap junction expression in the heart serves as a powerful factor for both the initiation and maintenance of arrhythmias (7, 15, 28). Areas of focal uncoupling, as demonstrated in the chimeric mouse, are associated with a significant increase in spontaneous ectopic events, possibly by unmasking intrinsic automaticity or by disrupting the conduction wave front leading to wave breaks. However, our results indicate that more extensive structural and/or functional remodeling may be required in the mouse heart to sustain ventricular arrhythmias.

Table 4. Electrocardiographic and electrophysiological data from Cx43−/− chimeric mice

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<th>Controls</th>
<th>Chimeras</th>
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<tr>
<td></td>
<td>n</td>
<td></td>
</tr>
<tr>
<td>PR interval, ms</td>
<td>40.8±1.02</td>
<td>37.2±1.42</td>
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<tr>
<td>QRS interval, ms</td>
<td>10.8±0.20</td>
<td>11.6±0.40</td>
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<tr>
<td>RR interval, ms</td>
<td>144.4±5.17</td>
<td>147.0±7.30</td>
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<tr>
<td>QRSA, mv</td>
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<td>0.095±0.009</td>
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<tr>
<td>VERP, 120, ms</td>
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<td>45.9±2.0</td>
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<tr>
<td>VERP, 100, ms</td>
<td>45.9±4.9</td>
<td>46.0±2.5</td>
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<tr>
<td>VERP, 80, ms</td>
<td>46.5±5.3</td>
<td>46.4±3.7</td>
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Values are means ± SE; n, number of mice; QRSA, QRS amplitude; VERP, ventricular effective refractory period.

ACKNOWLEDGMENTS

Blastocyst injection for the production of chimeric Cx43 knockout mice was performed in the Mouse Genetics Shared Resource Facility of the Mount Sinai School of Medicine, New York, NY. F. L. Chen is currently affiliated with the Department of Medicine, University of Pittsburgh School of Medicine, Pittsburgh, PA.

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