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Electrical remodeling contributes to complex tachyarrhythmias in connexin43-deficient mouse hearts

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Abstract

Loss of connexin43 (Cx43) gap junction channels in the heart results in a marked increase in the incidence of spontaneous and inducible polymorphic ventricular tachyarrhythmias (PVTs). The mechanisms resulting in this phenotype remain unclear. We hypothesized that uncoupling promotes regional ion channel remodeling, thereby increasing electrical heterogeneity and facilitating the development of PVT. In isolated-perfused control hearts, programmed electrical stimulation elicited infrequent monomorphic ventricular tachyarrhythmias (MVT), and dominant frequencies (DFs) during MVT were similar in the right ventricle (RV) and left ventricle (LV). Moreover, conduction properties, action potential durations (APDs), and repolarizing current densities were similar in RV and LV myocytes. In contrast, PVT was common in Cx43 conditional knockout (OCKO) hearts, and arrhythmias were characterized by significantly higher DFs in the RV compared to the LV. APDs in OCKO myocytes were significantly shorter than those from chamber-matched controls, with RV OCKO myocytes being most affected. APD shortening was associated with higher levels of sustained current in myocytes from both chambers as well as higher levels of the inward rectifier current only in RV myocytes. Thus, alterations in cell-cell coupling lead to regional changes in potassium current expression, which in this case facilitates the development of reentrant arrhythmias. We propose a new mechanistic link between electrical uncoupling and ion channel remodeling. These findings may be relevant not only in cardiac tissue but also to other organ systems where gap junction remodeling is known to occur.

Keywords

gap junction channels; ion channel remodeling

It is estimated that between 300,000 and 400,000 deaths in the United States each year result from sudden cardiac death, yet the electrophysiologic processes involved in the initiation and maintenance of complex ventricular tachyarrhythmias (VTs) remain largely unknown (1). Recent evidence has accumulated supporting the hypothesis that ventricular fibrillation (VF) demonstrates a high degree of spatiotemporal organization (2–6). Experimental studies have suggested polymorphic ventricular tachyarrhythmia (PVT) or VF can be maintained by a small number of high-frequency rotors that anchor in certain regions of the heart (5,6). Rotors have been shown to anchor in areas with shorter action potential durations (APDs) due to increased inward rectifier current (I_{K1}) allowing for higher excitation frequencies (7–9). Because rotors anchor in regions with relatively increased outward potassium current, gradients involving

repolarizing currents also may contribute to complex wave fractionation, which is characteristic of PVT. Thus, regional differences in cellular electrophysiological properties may play a central role in establishing the arrhythmogenic substrate (7,8).

We previously demonstrated that loss of the major ventricular gap junction protein connexin43 (Cx43) is associated with significant slowing of impulse conduction and a disruption of the ventricular activation pattern during sinus rhythm (10,11). Unlike wild-type mice, Cx43-deficient mice are highly susceptible to complex ventricular arrhythmias, making them a useful model to investigate arrhythmia dynamics and mechanisms of sudden arrhythmic death (10, 12). Given the propensity of these knockout mice to develop PVTs, we hypothesized that in addition to conduction slowing, loss of Cx43 may contribute to the arrhythmogenic substrate by secondarily increasing electrical heterogeneity. The purpose of this study was to first determine the spatiotemporal organization of ventricular arrhythmias in this model of sudden cardiac death. Second, we sought to determine whether loss of Cx43 is associated with chamber specific remodeling of conduction velocity (CV), APD, or the major repolarizing currents. Our studies demonstrate for the first time that loss of Cx43 contributes to the incidence of arrhythmias not only through conduction slowing but also through significant remodeling of the major outward potassium currents.

MATERIALS AND METHODS

Mice

All animal procedures were carried out in accordance with the Guide for the Care and Use of Laboratory Animals (U.S. National Institutes of Health Publication 86-23) and approved by the New York University School of Medicine Institutional Animal Care and Use Committee. Cardiac-restricted Cx43-deficient conditional knockout (OCKO) mice and 129/SvJ or littermate controls (CTLs) in which the Cx43 gene was “floxed” but that did not carry the Cre recombinase gene were used for this study (10,12). All mice studied were 2–5 months old.

Heart isolation and optical mapping

Mice were heparinized (500 U/kg) to prevent intracardiac blood coagulation, and euthanized with 100% carbon dioxide. Hearts were surgically removed *via* a thoracotomy. While fully immersed in oxygenated (95% O₂, 5% CO₂) Tyrode's (composition [mmol/L]: NaCl 114, NaHCO₃ 25, dextrose 10, KCl 4.6, CaCl₂ 1.5, Na₂PO₄ 1.2, MgCl₂ 0.7), the aorta was cannulated and Langendorff perfused at a constant pressure of 70 mmHg at 37°C. Hearts were allowed to recover for 20 min and then stained with the voltage-sensitive dye, Di-4-ANEPPS (Molecular Probes Inc., Eugene, OR, USA). High-resolution optical mapping experiments were performed as described previously (10,13–15). Recordings were made with a Dalsa CAD-128 CCD camera (Waterloo, ON, Canada) at 947 frames/s in bin mode (64×64 pixels) with 12-bit resolution in the absence of any pharmacological or mechanical motion-reduction techniques.

Epicardial CV

Epicardial CV measurements were obtained by pacing the right ventricle (RV) or left ventricle (LV) directly with a platinum monopolar electrode at a basic cycle length (BCL) of 100 ms using 4 ms stimuli at 2× diastolic threshold. Activity was recorded for 2 s. Post acquisition processing of optical movies included digital filtering to remove camera noise and signal-averaging to improve the signal to noise of the recordings. The filter used was a digital 60 Hz notch filter, and signal averaging was performed using at least 20 beats at the pacing frequency. CVs were calculated as described previously (13,15,16).

Arrhythmia induction and analysis

Induction of arrhythmias in CTL hearts was achieved using a custom ramp pacing protocol. The pacing protocol consisted of stepwise reductions in BCL, with 700 stimuli delivered at each BCL. Pacing was started at a BCL of 100 ms and reduced in 10 ms intervals until either capture was lost or a sustained arrhythmia was induced. This process was repeated up to 3 times. Stimuli were $2\times$ diastolic threshold and 1 ms in duration. Using this protocol, ventricular arrhythmias were induced in 4 of 8 CTL hearts. These arrhythmias were all characterized by stable, repetitive patterns of activation. In 2 hearts, arrhythmic episodes lasted long enough to capture activity from the anterior view.

In all cases, arrhythmias in OCKO mice spontaneously developed. Arrhythmias lasting more than 30 s were considered sustained. Once initiated, the pattern of activation remained stable throughout the experiment. Two-second movies of electrical activity were sequentially recorded from the anterior, posterior, RV, and LV free walls. The power spectrum of the time series for each pixel was calculated using the fast Fourier transform (FFT). The frequency with the maximum power was considered to be the dominant frequency (DF). Comparisons of RV and LV frequencies were performed in a pairwise fashion from the same heart. Arrhythmic episodes were successfully recorded from both the RV and LV surfaces in a total of 11 hearts; monomorphic ventricular tachyarrhythmia (MVT) was recorded in 6 hearts, and PVT was recorded in 5 hearts. No CTL or Cx43-OCKO hearts were excluded from any subset analysis.

Whole cell patch clamp studies

Myocytes were enzymatically isolated from the RV and LV free walls of 6- to 12-wk-old CTL and OCKO hearts. Membrane capacitance and series resistance were compensated electronically. Only cells with seal resistances $>1\text{ G}\Omega$ were included. Action potentials (APs) were initiated with a brief current pulse (2 ms; $2\times$ diastolic threshold). Measurements were made following 10 s of pacing at 2 Hz. For all experiments, the ionic composition of the external solution was (in mM): 137 NaCl, 4 KCl, 1.8 CaCl_2 , 1.2 MgCl_2 , 11 dextrose, 10 HEPES, adjusted to a pH of 7.4 with NaOH. The composition of the patch pipette was (in mM): 130 KCl; 1 MgCl_2 , 5 NaATP, 7 NaCl, 5 EGTA, 5 HEPES, adjusted to a pH of 7.2 with KOH. The transient outward current (I_{to}) was measured as the difference of the peak current (obtained with and without an inactivating prepulse of 100 ms to -40 mV) elicited by 500 ms voltage steps from -90 to 50 mV in 20 mV increments from a holding potential of -80 mV . Sustained current (I_{sus}) was measured as a current present at the end of the 500 ms voltage pulse (17). I_{K1} was measured using 500 ms voltage steps from -140 to -40 mV in 10 mV steps from a holding potential of -80 mV (17,18).

Statistical analysis

Data are presented as mean $\pm$ se unless otherwise indicated. Mean values were compared with either Student's t test or a single-factor ANOVA. Paired Student's t test was used to compare RV and LV frequencies recorded from the same heart. Post hoc Student's t tests were performed when significant differences were detected.

RESULTS

Impulse conduction and arrhythmia dynamics in CTL hearts

Studies in our lab as well as others have demonstrated that the murine heart is relatively resistant to arrhythmias. To determine the dynamics of arrhythmias in CTL mice, we developed a ramp pacing protocol that is capable of inducing ventricular arrhythmias in CTL mice. Using this protocol, we successfully induced VTs in 4 of 8 hearts. In 2 of these 4 hearts, only nonsustained (lasting $<30\text{ s}$) arrhythmias were observed; in the other 2 hearts, sustained arrhythmias were

recorded. Both sustained and nonsustained arrhythmias were monomorphic, characterized by stable repetitive patterns of activation. PVT was never observed. Figure 1 shows the pattern of activation recorded during one episode of sustained MVT. Fig. 1A is the color activation map recorded from the anterior view; Fig. 1B is the pseudoelectrocardiogram (pseudo-ECG); and Fig. 1C is the FFT. The dominant frequency of this arrhythmia was 28 Hz. On average, dominant frequencies for sustained and nonsustained arrhythmias were 25 ± 1.5 Hz ($n=4$).

We hypothesized that, in addition to the small size of the murine heart, protection from complex polymorphic arrhythmias may result in part from relatively homogenous electrophysiological parameters. To test this hypothesis, we measured CVs, APDs, and potassium channel currents in the RV and LV. Figure 2A, B shows CV and anisotropic ratio measurements obtained by pacing the RV and LV in CTL hearts. We did not find significant differences between the RV and LV with respect to conduction velocities (0.42 ± 0.03 and 0.35 ± 0.02 mm/ms; $P=0.08$) or anisotropic ratios (1.72 ± 0.12 , 1.83 ± 0.06 in the RV and LV, respectively; $P=0.43$). Fig. 2C, D shows a comparison of action potentials measured from isolated RV and LV myocytes. APs were not significantly different with respect to resting membrane potential (RMP), action potential amplitude (APA), or APD. Table 1 summarizes the APA and RMP measurements for the CTL hearts.

There have been reports of regional and chamber-specific differences in the level of I_{to} (19, 20). Thus, we sought to confirm the extent of these differences in our CTL hearts. A summary of the major potassium currents recorded from isolated RV and LV CTL myocytes is shown in Fig. 3. Representative current traces used to measure I_{to} and I_{sus} from RV and LV myocytes are shown in Fig. 3A, B, respectively. Fig. 3C shows measurements of I_{to} from the RV and LV of CTL hearts. Consistent with other reports (19), higher levels of I_{to} were found in RV compared to LV cells. However, this difference is not large enough to significantly impact the APD of RV myocytes. Similarly, as shown in Fig. 3D, I_{sus} levels were not significantly different between the ventricular chambers. I_{K1} is important in determining both the RMP and the late phase of repolarization (21). Representative current traces used to measure I_{K1} from RV and LV myocytes is shown in Fig. 3E, F, respectively. In addition, previous studies have suggested that I_{K1} is an important determinant of arrhythmia stabilization (7,8). Fig. 3G shows RV and LV measurements of I_{K1} from CTL myocytes. Similar levels of I_{K1} were found in both RV and LV CTL myocytes. In summary, our ramp pacing protocol was successful at inducing sustained MVTs with relatively homogenous frequency characteristics. The cellular electrophysiology of CTL hearts suggests they lack significant gradients of CV, anisotropic ratio, APD, or ion channel function.

Impulse conduction and arrhythmia dynamics in OCKO hearts

Our previous studies have demonstrated that loss of Cx43 in the myocardium is associated with a significantly higher propensity to develop sustained VTs (10). A total of 27 OCKO hearts were included in the arrhythmia study. We induced sustained VT in the majority of these hearts (18 of 27) and were able to record electrical activity from multiple surfaces during the arrhythmia in 11 of these 18. MVT, characterized by repetitive activation patterns with no regional differences in dominant frequencies (DFs) was observed in 6 of the 11 hearts. Figure 4 shows an example of sustained MVT where a stable rotor was located on the anterior surface. Other regions of the heart were able to follow the source in a 1:1 fashion. The pseudo-ECG shows a highly periodic signal with a cycle length of ~ 35 ms. The FFT indicates the DF of this arrhythmia was 27 Hz. In each heart with MVT, the pattern and location of the arrhythmia remained stable throughout the experiment; however, the source varied from heart to heart and was found on both the LV and RV free walls.

Sustained PVT was observed in 5 of the 11 OCKO hearts that were optically mapped and was characterized by beat-to-beat changes in the pattern of activation resulting in regional DF

differences. Figure 5 shows DF maps obtained from the RV and LV free walls recorded during PVT. The RV DF map indicates the RV was uniformly activated at ~33 Hz. The LV map shows slower and regionally specific differences in DFs. Regions near the base of the LV were activated at a similar frequency as the RV; however, the central region was activated more slowly. Differences in the rate of activation also were evident in the pseudo-ECG recordings and their power spectra. All of the RV DF maps were characterized by higher and more uniform frequencies, whereas the LV maps showed slower and more varied patterns ($n=5$).

Figure 6 shows a summary of the RV and LV frequency characteristics of the MVT and PVT. During MVT, the RV and LV DFs within individual OCKO hearts were compared pairwise and were not significantly different. On average, the DFs were 27.3 ± 1.7 , 26.8 ± 1.3 Hz in the RV and LV, respectively ($n=6$; $P=0.84$). Interestingly, these frequencies were similar to those recorded from littermate CTLs (compare to Fig. 1). During PVT, DFs also were compared pairwise and showed chamber-specific differences. The average DFs in the LV were 26 ± 2.5 Hz, whereas the RV DFs were 33 ± 2.5 Hz, representing a significant increase of DFs in the RV ($n=5$; $P=0.016$). Note that similar LV DFs occurred during both MVT and PVT, suggesting this is the maximum DF possible in the LV. In all hearts where PVT occurred, higher DFs were found in the RV compared to the LV.

Regional potassium channel remodeling in OCKO hearts

One possible explanation for regionally specific differences in DFs during PVT is differences in epicardial CVs. Although we have previously shown significant slowing of conduction in Cx43-deficient hearts, comparative analysis of CVs in the RV and LV has not been performed. Figure 7A, B shows CV and anisotropic ratio measurements obtained by pacing the RV and LV in OCKO hearts. Similar to CTL hearts (Fig. 2), we did not find significant differences when RV and LV conduction velocities were compared in OCKO mice (0.23 ± 0.02 , 0.21 ± 0.02 in the RV and LV, respectively; $P=0.33$). In addition, similar anisotropic ratios were found also in both the RV and LV (2.05 ± 0.22 , 2.09 ± 0.19 in the RV and LV, respectively; $P=0.9$). These data suggest that regional differences in CVs or anisotropic ratios do not account for the higher RV activation frequencies during PVT.

Next, we evaluated AP parameters in isolated OCKO myocytes, as shown in Fig. 7C, D. In contrast to CTL hearts (see Fig. 2), APD_{50} , APD_{70} , and APD_{90} recorded from RV OCKO myocytes were significantly shorter compared to the LV OCKO myocytes. Table 1 summarizes the APA and RMP measurements for the RV and LV of CTL and OCKO hearts. In addition to the shorter APD in the RV of OCKO hearts, the RMP of these cells was significantly hyperpolarized compared to the RV CTL cells. In summary, a left-to-right APD gradient was found only in OCKO hearts and resulted from shortening of APD in the RV.

We hypothesized that shorter RV APDs were due to remodeling of certain repolarizing currents. Previous investigators have shown that I_{to} often is modulated in models of cardiac disease (22–24). In addition, there have been reports of regional and chamber-specific differences in the level of I_{to} (19,20). Thus, we sought to determine whether changes in I_{to} contributed to RV APD shortening in OCKO hearts. Figure 8A shows measurements of I_{to} from the RV and LV of OCKO hearts. Interestingly, unlike CTL hearts, similar levels of I_{to} were found in RV compared to LV cells. Thus, remodeling of I_{to} would not explain the gradient of APD or the chamber-specific differences in the DFs. Next, we looked at I_{sus} levels in RV and LV myocytes. Higher outward I_{sus} levels were found in the RV compared to the LV myocytes at +30 and +50 mV. Moreover, overall higher I_{sus} levels were found in the OCKO compared to CTL myocytes (Fig. 2B). Since this increase occurred at relatively positive potentials with respect to the cardiac action potential, the overall effect would be a relatively modest shortening at all levels of repolarization. In addition to changes in I_{sus} levels, we also found significant remodeling of I_{K1} . I_{K1} is important in determining both the RMP and the late

phase of repolarization (21) and may be an important determinant of arrhythmia stabilization (7,8). Figure 8C shows RV and LV measurements of I_{K1} from OCKO myocytes. Significantly higher levels of I_{K1} were found in the RV, compared to LV, OCKO myocytes. These data suggest that significant remodeling of I_{K1} occurs in the RV of OCKO hearts. Although the zero crossover point was not negatively shifted, the changes in I_{K1} levels are consistent with APD shortening and a more negative RMP. Increased I_{sus} levels would supply additional outward current, providing a mechanistic explanation for the shorter APDs observed in the OCKO myocytes, whereas the chamber-specific differences in I_{K1} levels would account for regional APD shortening in the OCKO RV myocardium.

DISCUSSION

We have shown previously that cardiac-specific loss of Cx43 is associated with significant conduction slowing and a higher propensity for the development of ventricular arrhythmias (10,12). In the present study, in an effort to mechanistically link a primary perturbation of gap junction function with this highly arrhythmic phenotype, we have investigated conduction properties, arrhythmia dynamics, and cellular electrophysiological parameters in OCKO hearts and myocytes. First, we demonstrated that during MVT, similar DFs were found in both the RV and LV myocardium. At these slower rates of activation, both the RV and LV were capable of maintaining the arrhythmic source. Second, during PVT, significantly higher DFs were found in the RV compared to the LV. In all cases of PVT, DFs in the RV were uniform, suggesting a single activation rate, whereas activation on the LV was slower and more fractionated. Third, conduction velocities were slowed equally in both ventricular chambers. Thus, CVs do not account for the chamber-specific differences in PVT dynamics. Fourth, cardiac-specific deletion of Cx43 is associated with significant shortening of APD in the RV. Interestingly, patch clamp measurements demonstrated that loss of Cx43 is associated with functional remodeling of the major potassium current involved in cardiac repolarization, including I_{to} , I_{sus} , and I_{K1} . Specifically, higher levels of I_{sus} and the outward component of I_{K1} in the RV provide a mechanism for APD shortening and chamber-specific differences in PVT dynamics.

Gap junction remodeling is a common response to many forms of heart disease. Previous studies from our group and others have demonstrated that loss of cell-cell coupling is highly arrhythmic; however, a detailed understanding of arrhythmia dynamics has been lacking (10, 12,25–28). In the normal mouse heart, sustained MVT can be induced with burst pacing, although PVT is exceedingly rare (29). Here, we have developed a more reliable method for inducing ventricular arrhythmias in CTL hearts. Using this protocol, we were able to systematically measure arrhythmia frequencies during MVT. Interestingly, rates of MVT observed in the CTL hearts were similar compared to the OCKO hearts. Although diminished electrotonic coupling might be expected to slow CV and lower activation rates, loss of Cx43 dramatically increased the susceptibility to PVT, and these tachycardias displayed even higher rates. Our study suggests that electrical remodeling, specifically increased levels of I_{K1} and I_{sus} , lead to a reduction in APD in the RV and may contribute to this behavior. In addition to contributing to the shorter APDs, higher levels of I_{K1} are predicted to have a hyperpolarizing effect on the RMP. We speculate that these changes in action potential characteristics may increase the availability of sodium current by allowing for faster recovery from inactivation. Although this effect may be minimal at physiological rates, the enhanced availability of sodium current may become more prominent at faster activation rates. These alterations would influence core diameter and interactions of the wavefront and wavetail, facilitating the higher frequencies we observed in Cx43-deficient hearts (7). Consistent with our results, van Rijen *et al.* (27), using an alternative model of Cx43 deficiency, also observed PVT originating from the RV, although their reported cycle lengths were substantially slower (60–80 ms) compared to the arrhythmias reported here (~30 ms). We would predict also, as potassium currents

remodel, the likelihood for polymorphic arrhythmias would increase. Because the hearts used for our patch clamp analysis were, for technical reasons, not selected *a priori* based on arrhythmic morphology, our measurements of current density likely underestimate the true extent of remodeling in response to Cx43 deficiency.

Several factors are known to be important in determining whether a rotor will drift or become anchored (3,30), including anatomical obstacles, the development of fibrosis, scars that form during myocardial infarction, and differing levels of ionic currents. Recent experiments from the Jalife group (7,8) have suggested that rotor anchoring is associated with higher levels of the outward component of I_{K1} in the anterior LV. This group has argued that I_{K1} contributes outward current during late repolarization; thus, increased levels of I_{K1} would lead to shorter APDs, which in turn would allow for faster rates of activation. Wave breakup would occur as impulses moved into regions of lower I_{K1} expression. However, other structural and electrophysiological differences between the ventricular chambers, including wall thickness, gradients of CV, and other membrane currents, also could contribute to arrhythmia stabilization. In this study, high-frequency arrhythmic sources were found in the RV instead of the LV. The fact that higher DFs were found in the chamber with functionally higher levels of I_{K1} provides further evidence that I_{K1} current plays a major role in determining the location of arrhythmic sources.

We have recently shown that the pattern of activation during sinus rhythm is significantly disrupted in OCKO hearts (11). In CTL mice, normally there are 2 epicardial breakthrough sites near the apex of the heart that initiate ventricular activation. Sinus rhythm activation patterns in OCKO hearts are characterized by multiple independent sites of breakthrough activation that occur throughout the ventricles (11). We argued that this is the result of reduced coupling in the muscle, allowing for propagation at normally silent Purkinje muscle junctions. Other laboratories have suggested that altering either the rate or the pattern of activation is associated with long-term electrical remodeling of the myocardium (31,32). Indeed, disruption of the normal ventricular activation sequence has been shown to lead to significant electrical remodeling, raising the possibility of important secondary effects resulting from altering connexin levels. In addition to changing levels of I_{to} and I_{K1} , remodeling of other membrane ionic currents also may occur, including sodium and calcium currents. Thus, one interesting potential explanation for remodeling of ionic currents in OCKO hearts could be directly related to the altered ventricular activation pattern. Interestingly, the disruption of sinus activation patterns also may play an important role in induction of cardiac arrhythmias. The multisite sinus activation patterns would create conditions in which a high number of wavefronts would collide. Similar to arrhythmia induction with cross-field stimulation protocols, wavefront collisions, if timed properly, can lead to the formation of vortices.

One of the most interesting and important findings made from connexin-deficient mice is the observation that unexpectedly large reductions of connexin expression levels are required to significantly affect epicardial conduction velocities. We have previously reported a 50% reduction of Cx43 levels do not produce significant conduction slowing (15). That report and others have demonstrated reductions of more than 95% are required to produce a 50% slowing of epicardial conduction. This study is the first to demonstrate that conditional inactivation of Cx43 is associated with a high degree of regionally specific electrical remodeling within the myocardium. We found increased levels of I_{K1} in the RV resulted in shorter APDs, which in turn allowed for higher rates of activation during PVT.

While our study identifies a key interaction between passive junctional and active sarcolemmal ionic currents, recent data suggest that altered connexin expression may more broadly affect cellular and organ-level behavior. For example, Spray *et al.* (33) have identified hundreds of transcripts that are altered in abundance in the hearts of Cx43 knockout mice compared to wild-

type CTLs, including such key regulatory proteins as the β_2 adrenergic receptor. Similarly, there are major alterations in transcriptional behavior in the brains of Cx43-deficient mice (34,35). Thus, taken together, these studies highlight the important influence of connexins and gap junction remodeling on broad aspects of physiology and pathophysiology.

Study limitations

There are several limitations to this study that should be mentioned. First, optical maps of arrhythmias taken from four views were obtained sequentially, not simultaneously; thus, in theory, arrhythmias could have drifted during the mapping procedure. However, in most cases, each view was mapped more than once to confirm the stability of arrhythmia and DF patterns. Second, we were not able to map the interventricular septum. It is possible that in some cases we did not map the entire reentrant circuit since the arrhythmic source may have been partially or fully located on the endocardial surface or interventricular septum. In these cases, higher-frequency sources of activity originating from this region could not be excluded. Third, comparison of RV and LV refractory periods in CTL and OCKO hearts was not attempted, given the propensity for induction of VT when pacing near the refractory period. Moreover, differences in effective refractory periods were expected to be quite small and would likely require a large number of animals to achieve statistical significance. Finally, we could not correlate current density and arrhythmic vulnerability in the same hearts, although, as noted above, this limitation would tend to underestimate the magnitude of electrical remodeling.

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Figure 1. MVT in CTR mice. *A)* Activation map recorded during MVT from the anterior view. *B)* Pseudo-ECG shows stable repetitive activity. *C)* FFT of the pseudo-ECG shows the power spectra and a dominant frequency of 28 Hz.

Figure 2.

Regional electrophysiological characteristics of control mice. CV measurements from the LV and RV of CTL hearts are shown. *A*) Anisotropic ratio measured in the RV and LV. *B*) No significant differences with respect to CV or anisotropic ratios between the RV and LV were found. *C*) APs recorded from right (dashed line) and left (solid line) ventricular myocytes isolated from CTL. *D*) Comparison of APDs measured from RV and LV myocytes of CTL myocytes.

Figure 3.

Potassium current levels recorded from CTL RV and LV myocytes. *A, B*) Representative current traces from RV (*A*) and LV myocytes (*B*). Insets show voltage clamp protocols. *C*) Peak I_{to} recorded from the RV and LV. Peak outward I_{to} densities in CTL myocytes are higher in the RV compared to the LV. *D*) I_{sus} levels recorded from RV and LV myocytes are similar. *E, F*) Representative I_{K1} current traces from RV (*E*) and LV myocytes (*F*). *G*) I_{K1} levels measured in the RV and LV of CTL hearts.

Figure 4.

MTC in OCKO mice. *A–D*) Activation maps of an MVT recorded from the RV (*A*), anterior (*B*), LV (*C*), and posterior views (*D*). The reentrant source can be seen on the anterior surface, with the center of rotation positioned near the junction of the RV and LV (*). *E*) Pseudo-ECG obtained from the movie shown in *A*, showing stable monomorphic activity. *F*) Pseudo-ECG obtained from the movie shown in *C*. *G*) Power spectrum of the movie shown in *A* shows a highly periodic signal with a dominant frequency at 27 Hz. *H*) Power spectrum obtained from the movie shown in *C*. Color scale indicates 0–35 ms for *A* and *C*; 0–22 ms for *B* and *D*.

Figure 5.

Dominant frequency maps of the RV and LV during polymorphic VT in OCKO mice. *A, B*) DF map obtained during PVT from the RV (*A*) and LV (*B*). *C, D*) Pseudo-ECG recordings obtained from the RV (*C*) and LV (*D*). *E, F*) Power spectra of the pseudo-ECG for the RV (*E*) and LV (*F*).

Figure 6.

RV- and LV-dominant frequencies recorded during monomorphic (A) and polymorphic (B) arrhythmias. During MVT, the average dominant frequency was not significantly different between the RV and LV (RV $DF=27.3 \pm 1.7$; LV $DF=26.8 \pm 1.3$; $n=6$; $P=0.84$). Similar dominant frequencies were recorded from the LV during PVT; however, significantly higher dominant frequencies were recorded in the RV (RV $DF=33 \pm 2.5$; LV $DF=26 \pm 2.5$; $n=5$; $P=0.016$).

Figure 7.

RV remodeling in OCKO hearts. *A*) LV and RV CV measurements in OCKO hearts. CV was reduced by ~50% in both the RV and LV of OCKO mice compared to CTLs (Fig. 2). *B*) Anisotropic ratios in the LV and RV of OCKO hearts. However, no significant differences with respect to CV or anisotropic ratios between the RV and LV were found. *C*) Examples of Aps recorded from right (dashed line) and left (solid line) ventricular myocytes isolated from OCKO hearts. *D*) Comparison of APDs measured from right and left ventricular myocytes of OCKO hearts. Significant differences in APDs measured at 50, 70, and 90% repolarization were found in the OCKO myocytes.

Figure 8.

Remodeling of potassium currents in the OCKO RV myocytes. *A, B*) Representative current traces from RV (*A*) and LV myocytes (*B*). Insets show voltage clamp protocols. *C*) I_{to} levels in the RV and LV. *D*) I_{sus} levels recorded from RV and LV myocytes. *E, F*) Representative I_{K1} current traces from RV (*E*) and LV myocytes (*F*). *G*) I_{K1} levels recorded from RV and LV myocytes.

TABLE 1

RMP and APA measurements in CTL and OCKO hearts[1]

Measure	CTL		OCKO	
	RV (n=13)	LV (n=10)	RV (n=14)	LV (n=17)
RMP, mV	-76.5 ± 0.8	-76.2 ± 0.9	$-81.0 \pm 0.8^*$	-79.0 ± 0.7
APA, mV	127 ± 2.2	123 ± 3.5	129.1 ± 1.5	125.9 ± 2.5

* $P < 0.05$ vs. CTL RV.