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Reduced Heterogeneous Expression Of Cx43 Results In Decreased Nav1.5 Expression And Reduced Sodium Current Which Accounts For Arrhythmia Vulnerability In Conditional Cx43 Knockout Mice

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Abstract

Background—Reduced Connexin43 (Cx43), sodium channel (Nav1.5) expression and increased collagen expression (fibrosis) are important determinants of impulse conduction in the heart.

Objective—To study the importance and interaction of these factors at very low Cx43 expression, inducible Cx43 KO mice with and without inducible ventricular tachycardia (VT) were compared by electrophysiology and immunohistochemistry.

Methods—Cx43^{CreER(T)/fl} mice were induced with Tamoxifen and sacrificed after 2 weeks. Epicardial activation mapping was performed on Langendorff-perfused hearts, and arrhythmia vulnerability was tested. Mice were subdivided in VT+ (n=13) and VT– (n=10) and heart tissue was analyzed for Cx43, Nav1.5 and fibrosis.

Results—VT+ mice had decreased Cx43 expression with increased global, but not local, heterogeneity of Cx43, compared to VT– mice. Nav1.5-immunoreactive protein expression was reduced in VT+ versus VT– mice, specifically at sites devoid of Cx43. Levels of fibrosis were similar between VT– and VT+ mice. QRS-duration was increased and epicardial activation was more dispersed in VT+ mice than in VT– mice. The effective refractory period (ERP) was similar between both groups. Premature stimulation resulted in a more severe conduction slowing in VT+ compared to VT– hearts in the right ventricle. Separate patch clamp experiments in isolated rat ventricular myocytes confirmed that loss of Cx43 expression correlated with decreased sodium current amplitude.

Conclusions—Global heterogeneity in Cx43 expression and concomitant heterogeneous downregulation of sodium channel protein expression and sodium current leads to slowed and dispersed conduction, which sensitizes the heart for ventricular arrhythmias.

Keywords

Cx43; Nav1.5; heterogeneity; sodium current; arrhythmia

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Introduction

A reduced expression of the main ventricular gap junction protein in the heart, Connexin43 (Cx43), is commonly found in a variety of cardiac pathologies, such as ischemia, hypertrophy and heart failure^{1,2}. The loss of Cx43, together with increased collagen deposition^{3,4} and a decreased expression of the cardiac sodium channel Nav1.5^{5,6}, are thought to impair proper conduction of the electrical impulse, increasing the risk for fatal ventricular arrhythmias⁷. Besides a decreased expression, a more inhomogeneous distribution of Cx43 has been found in remodeled hearts, resulting in a more dispersed conduction, which is also correlated with an increased susceptibility for arrhythmias^{8–11}.

Previous studies have shown that a 50% reduction in Cx43 expression mice did not affect impulse conduction^{12,13}. However, further reduced Cx43 levels by conditional deletion of the Cx43 gene to <5%, resulted in a high vulnerability for arrhythmias due to slowed and dispersed conduction^{13,14}. It was then proposed¹³, as shown in a genetic model of heterogeneous Cx43 expression⁸, that decreased and heterogeneous expression of Cx43 protein levels allowed for the occurrence of ventricular arrhythmias.

Despite the fact that genetic tools allow for specific down-regulation of Cx43, recent studies have shown that the intercalated disc is a highly dynamic structure with interaction between the different proteins that are located at the disc and that determine impulse conduction. A regulatory mechanism for Nav1.5 was shown to be localized at the intercalated disc, and Nav1.5 and Cx43 can be co-immunoprecipitated, suggesting an interaction at the level of the intercalated disc^{15,16}. Furthermore, *in vitro* experiments on cultured cardiomyocytes have shown that loss of Plakophilin-2 results in both Cx43 remodeling and decreased sodium current (I_{Na})^{17–19}. These studies point to a close relationship between Cx43 and Nav1.5, suggesting that proper expression and functioning of one protein is essential for the other.

In the present study, we focused on the factors that are responsible for the vulnerability to arrhythmias in mice with very low (<5%) Cx43 expression. We hypothesized that specifically in arrhythmogenic animals, extremely low expression levels of Cx43 can be found, with regions completely devoid of Cx43, resulting in slow and dispersed conduction. Secondly, given the interactions between Cx43 and the voltage gated sodium channel complex¹⁹, we speculated that diminished Cx43 protein levels may affect the abundance, distribution and/or function of Nav1.5 in these animals, further contributing to conduction disturbances. For this purpose, we subdivided Tamoxifen-induced Cx43^{Cre-ER(T)/fl} mice into arrhythmogenic (VT+) and non-arrhythmogenic (VT–) animals, and compared conduction parameters and tissue characteristics. Our data show that VT+ mice have a more severe reduction in Cx43 protein levels compared to VT– mice, which is accompanied by a global, but not local, heterogeneity in Cx43 expression. Furthermore, the abundance of the Nav1.5-immunoreactive protein was reduced in VT+ compared to VT– mice, whereas collagen content was similar. Separate studies showed that loss of Cx43 expression led to a decrease in the amplitude of I_{Na} in adult rat ventricular myocytes. Together, these combined changes resulted in dispersed conduction and severe conduction slowing during premature stimulation, making the heart highly prone to arrhythmias.

Materials and Methods

An expanded Materials and Methods section is available in the online data supplement. Brief descriptions are presented below.

Animals

Cx43^{Cre-ER(T)/fl} (n=17) mice were generated and injected with Tamoxifen as described previously¹³ and analyzed 13–15 days after the first injection. Extended analysis of previous data (n=15) on similar Tamoxifen-induced Cx43^{Cre-ER(T)/fl} mice, were included in this study¹³.

Preparation of the Hearts and Ventricular Conduction

ECG-measurements are Langendorff-experiments, as well as data analysis, are described in the online data supplement.

Statistics

The statistical tests used are described in the online data supplement.

Immunohistochemistry and Histology

After electrophysiological measurements, hearts were rapidly frozen in liquid nitrogen and stored at -80°C . For sectioning, (immuno-) labeling and quantification of Cx43 and fibrosis, see the online data supplement.

To quantify heterogeneity of Cx43 expression, photographs were transformed into 8-bit white (Cx43) and black pictures and subdivided in 140 equal squares. Of each square, the total intensity of Cx43 was determined. Micro heterogeneity was defined as the standard deviation of all squares divided by the mean in one photograph, whereas macro heterogeneity compared total intensities of all pictures of each ventricle of the heart. A detailed description can be found in the online data supplement.

For Nav1.5 quantification, at least 5 hearts of both groups were used. Of each heart, 3–5 randomly taken pictures were analyzed. Three blinded observers individually ranked all 45 pictures from high to low expression. Statistical analysis was performed on every ranking separately.

Determination of sodium current properties in Cx43-knockdown (Cx43-KD) cells

Cell-isolation, transfection and I_{Na} measurements were performed as described previously^{18,19}. See the online data supplement for a detailed description.

Results

Arrhythmogeneity

We analyzed 32 Tamoxifen-induced Cx43^{Cre-ER(T)/fl} mice, of which 9 died within 2 weeks after induction. Of the remaining 23 mice, 13 mice were susceptible to arrhythmias during Langendorff perfusion (57%). Five mice showed spontaneous arrhythmias and in 8 other hearts, arrhythmias were induced by either premature stimulation or burst pacing (Figure 1C). The majority of arrhythmias (10 out of 13) were sustained (>15 complexes followed stimulation). The other arrhythmias were non-sustained (~8 complexes followed stimulation, 1 heart) or premature beats (2 hearts). In the remaining 10 Tamoxifen-induced Cx43^{Cre-ER(T)/fl} mice, no arrhythmias could be induced.

Figure 1A shows a typical example of a spontaneous started polymorphic ventricular tachycardia. The activation maps of this pVT (lower panels) show irregular activation patterns. In contrast, epicardial electrograms of an induced arrhythmia in another heart showed a monomorphic ventricular tachycardia, with comparable activation patterns of consecutive beats (Figure 1B).

Ventricular conduction

Mice were divided in arrhythmogenic (13 mice, VT+) and non-arrhythmogenic (10 mice, VT-) animals to determine factors that are associated with the susceptibility for arrhythmias. No differences in induction-time, heart weight / body weight (HW/BW) ratio or gender distribution were found between the two groups (Table 1).

QRS-duration was higher in VT+ compared to VT- mice (15.4 ± 1.1 ms vs 12.1 ± 0.3 ms respectively, Table 1). The left panel in Figure 2 shows representative epicardial activation maps of Cx43^{Cre-ER(T)/fl} VT- and VT+ hearts of both LV and RV during stimulation at a BCL of 120ms. Stimulation from the center of the grid resulted in anisotropic activation patterns determined by the fiber direction. No significant differences in impulse conduction velocity longitudinal (CV_L) and transverse (CV_T) to the fiber orientation were found between the groups, although conduction was generally somewhat slowed in VT+ compared to VT-. Concomitantly, the anisotropic ratio (CV_L/CV_T) in LV and RV were not different between both groups (Table 1). However, VT+ hearts showed regions with crowding of isochrones, indicative of local conduction slowing. Therefore, we determined the dispersion of conduction index²⁰. We found higher values of dispersion in VT+ compared to VT- mice, which was significant in RV with a near-significant similar trend in LV ($p=0.058$).

The right panel in Figure 2 shows epicardial activation after premature stimulation (ERP +5ms). Conduction slowing both longitudinal and transverse to the fiber direction was more prominent in VT+ compared to VT- hearts, which was significant for CV_L and CV_T in RV (Table 1). The effective refractory period (ERP) was similar in both LV and RV for Cx43^{Cre-ER(T)/fl} hearts VT- and VT+ (Table 1).

Cx43 expression and heterogeneity

Figure 3 shows typical examples of Cx43-immunolabeled sections of Tamoxifen-induced Cx43^{Cre-ER(T)/fl} VT- and VT+ hearts. As expected, Tamoxifen-induction resulted in a very low Cx43 expression in both LV and RV, while N-cadherin (a highly stable component of the adherens junction in the ID) was still abundantly present at the intercalated discs. Whereas Cx43 was detectable throughout the ventricles in VT- mice, large areas in VT+ hearts showed a complete depletion of Cx43 expression (lower panels VT+ in Figure 3), with some local spots, positive for Cx43 (upper panels VT+ in Figure 3). As a result, total Cx43-immunosignal was decreased in VT+ compared to VT- hearts in both LV and RV (Table 2).

Next, we checked whether expression of Cx43 was more inhomogeneous in VT+ compared to VT- hearts. We first analyzed local Cx43 heterogeneity by quantifying intensity differences in Cx43 expression within pictures. Table 2 shows that this micro heterogeneity of Cx43 expression was similar between VT+ and VT- hearts in both LV and RV. However, macro heterogeneity of Cx43 expression, which quantifies the differences among regions of one complete ventricle of a heart, was significantly increased in both LV and RV in VT+ compared to VT- hearts (Table 2).

Nav1.5 expression and fibrosis

We further analyzed the cardiac tissue on other determinants of conduction, and stained for the cardiac sodium channel Nav1.5. As can be appreciated from Figure 4A, the intensity of the Nav1.5 immunoreactive signal was reduced in VT+ compared to VT- hearts, while N-cadherin staining was comparable. To quantify these differences, three blinded observers ranked all pictures from high to low expression, and three independent Wilcoxon Rank Sum tests were performed. Table 2 shows that the average rank of pictures of VT- hearts was significantly higher compared to pictures of VT+ hearts for all observers ($p<0.001$ for each

individual ranking, average ranking of the three observers was 15.71 ± 0.14 vs 30.62 ± 0.14 , respectively), showing quantitatively that Nav1.5 expression was decreased in VT+ compared to VT- hearts.

To identify the possible correlation between heterogeneous Cx43 and Nav1.5 expression, we performed a double labeling for both proteins. As shown in figure 4B, staining for both Cx43 and Nav1.5 was quite homogeneous in VT-, whereas in VT+ hearts, heterogeneous expression of Cx43 colocalized with heterogeneous expression of Nav1.5. In regions positive for Cx43 expression, Nav1.5 expression was present as well (arrows), while in regions devoid of Cx43, Nav1.5 expression was also absent (asterisk).

Finally, we determined the presence of fibrosis by histochemical analysis with Picrosirius Red staining. Figure 4C shows low amounts of interstitial collagen in both VT- and VT+ hearts. Quantification revealed no significant differences in collagen content between VT- and VT+ hearts (Table 2).

Sodium current in Cx43-deficient cardiac ventricular myocytes

The experiments described above led us to characterize sodium current properties in Cx43-deficient cells. Isolated, adult rat ventricular myocytes were exposed to siRNA for Cx43 (Cx43-KD) or to a non-silencing construct (Cx43- ϕ KD). As shown in Figure 5A, siRNA treatment caused a decrease in Cx43 abundance to near zero levels. As shown in Figure 5B, loss of Cx43 expression led to a significant decrease in the average peak sodium current density. Yet, voltage dependence of inactivation (Figure 5C), and time course of reactivation (Figure 5D), were not affected by the loss of Cx43 expression. Overall, these results support the notion of a cross-talk between Cx43 expression, and the level of functional sodium current in cardiac myocytes.

Discussion

The main finding of this study is that in the background of very low (~5%) Cx43 levels, differences in tissue characteristics determine arrhythmia susceptibility in individual mice: severely reduced Cx43 protein levels with large regions of total depletion of Cx43, leading to global, but not local, heterogeneity of Cx43 expression. In regions devoid of Cx43 expression, the expression of Nav1.5 was absent as well. Separate experiments showed that loss of Cx43 expression leads to a decrease in the amplitude of I_{Na} , recorded from isolated ventricular myocytes. These data suggest that heterogeneous and combined downregulation of Cx43 and Nav1.5 lead to slowed and dispersed conduction which results in an increased propensity to ventricular arrhythmias.

Cx43 expression and heterogeneity

The very low Cx43 expression levels in Cx43^{CreER(T)/fl} mice after Tamoxifen-induction are in agreement with previous studies^{13,21}. Here we showed that VT+ mice have decreased Cx43 protein levels compared to VT- mice, indicating that the actual reduction in Cx43 expression is an important arrhythmogenic factor. With regard to conduction reserve, which is defined as 'the ability of the heart to maintain near normal impulse conduction velocity in the background of moderate changes in tissue characteristics (up to a ~50% reduction in Cx43 or Nav1.5, or a small increase in collagen content (~3 times increase))', this might have important implications²². Several studies have shown that when this conduction reserve is reduced, the heart becomes susceptible for arrhythmias^{23,24}. Although conduction was considerably slowed in VT- mice compared to mice with normal Cx43 expression (Cx43^{fl/fl} mice) arrhythmias were absent¹³. Noteworthy, we found that the further reduction in Cx43 expression in VT+ mice was accompanied by an increased heterogeneity of Cx43. From our data we cannot determine whether the extremely low total Cx43 expression levels,

or the global heterogeneity, with regions of total depletion of Cx43, is the main arrhythmogenic factor. Concerning this vulnerability to arrhythmias, it is likely that also the concomitantly decreased expression of the cardiac sodium channel Nav1.5 plays a role. Interestingly, we previously showed that a combined 50% decrease of both Cx43 and Nav1.5 did not increase arrhythmia susceptibility²⁵. Apparently, a moderate reduction in both proteins did not exceed conduction reserve. However, a reduced Nav1.5 expression combined with the extremely low levels of Cx43 as found in VT+ mice in this study, presumably makes the heart highly vulnerable to arrhythmias.

In remodeled hearts, reduced Cx43 levels are generally accompanied by an increased heterogeneity of Cx43 expression^{1,2}. We and others have shown that arrhythmogeneity is highly associated with a heterogeneous distribution of Cx43, both in mice and in patients⁸⁻¹¹. Therefore, we thoroughly analyzed Cx43 heterogeneity in this study by distinguishing local from global inhomogeneity. We calculated micro heterogeneity as a measure for local heterogeneity of Cx43 expression, which quantified differences in Cx43 intensity within a small region of the heart. Interestingly, we found no differences in this micro heterogeneity between VT- and VT+ mice. In contrast, macro heterogeneity calculated variations in Cx43 intensities among different regions of the heart, which was significantly increased in VT+ compared to VT- mice. This indicates that in our study, global, rather than local, heterogeneity is associated with arrhythmia vulnerability. This implies that the large areas in which Cx43 is totally depleted (lowest pictures Figure 3) significantly contribute to the formation of an arrhythmogenic substrate.

Nav1.5 expression and fibrosis

Besides gap junctional coupling between cardiomyocytes, impulse conduction in the heart is also dependent on the expression and function of the cardiac sodium channel Nav1.5, and the tissue architecture, mainly determined by the amount of collagen. Therefore, we stained for Nav1.5 and found a reduced expression in VT+ compared to VT- mice. This raised the important question whether this reduction in Nav1.5 is a direct consequence of the very low Cx43 protein levels. This question was addressed by co-labeling Cx43 and Nav1.5 and showed that regions devoid of Cx43 were characterized by absence of Nav1.5, while regions with Cx43 labeling also showed Nav1.5 labeling. This indicates that reduction of Cx43 is connected to reduction of Nav1.5 expression. Previous studies have shown that Cx43 and Nav1.5 are both present in the intercalated disc, and that destabilization of the desmosome by a loss of Plakophilin-2 results in Cx43 remodeling as well as a decreased functioning of Nav1.5^{15,17,18}. We have also shown that stressing mice with reduced or mutated Nav1.5 by aging or pressure overload resulted in a decreased expression of Cx43^{26,27}. These studies show a close relation between those proteins, which suggest that the depletion of Cx43, as found in large areas throughout the hearts of VT+ mice, may directly affect the function and/or abundance of Nav1.5. On the other hand, Johnson et al showed that in isolated neonatal cardiomyocytes from Cx43 null mice, Nav1.5 expression and function was unaffected²⁸. We have directly shown in this study that downregulation of Cx43 leads to reduction of sodium current. However, the consequences of Cx43 depletion in isolated cells may differ from the effects in an intact heart i.e. by stretch on the cardiomyocytes. Besides, the effects of silencing of Cx43 in a developmentally established system, as described in this study, may differ from the implications of Cx43-depletion during development. Additional research is required to prove a direct link between the decreased Cx43 and Nav1.5 levels in VT+ mice.

Because fibrosis is an important parameter for impulse conduction, we checked whether extremely low levels of Cx43 would also increase fibroblast activity, leading to increased collagen deposition. However, we found only low levels of interstitial fibrosis, which was similar in VT- and VT+ mice, indicating that fibrosis did not play a role in determining arrhythmia vulnerability in this study.

Impulse conduction

ECG analysis and epicardial activation mapping was performed on VT+ and VT- hearts to determine the electrical effects of the heterogeneous reduction in Cx43 and decreased Nav1.5 expression. We found a significant increased QRS-duration in VT+ compared to VT- mice, although epicardial conduction during Langendorff-perfusion was not significantly slowed during S1S1 pacing. However, we showed that conduction was more dispersed (only significant for RV) in VT+ compared to VT- mice. It is likely that the global heterogeneity of Cx43 expression is mainly responsible for this dispersed conduction: conduction is slowed in the regions of Cx43-depletion, whereas it is relatively normal in parts that express Cx43. Challenging the hearts by premature stimulation resulted in a more severe conduction slowing in VT+ than in VT- hearts (only significant for RV). The decreased Nav1.5 availability in VT+ mice could play a fundamental role, since the upstroke velocity of the action potential is restricted by the number of active Nav1.5 channels, which is particularly crucial during premature stimulation. The slow impulse conduction during premature stimulation in VT+ mice enables the induction of ventricular tachycardias. Interestingly, we found no differences in ERP between VT- and VT+ mice, indicating that arrhythmia vulnerability was not caused by alterations in refractoriness.

Study limitations

In this study we used different methods to quantify Cx43 and Nav1.5 expression. Because of the relatively high background staining in Nav1.5 labeled sections, we could not apply a standardized method by Image J. Therefore, pictures were ranked by 3 blinded observers and statistically analyzed by a Wilcoxon Ranked Sum Test. The variance among the observers was very low, and statistical analysis of all 3 individual tests showed a significant difference between VT+ and VT- mice, indicating a very high reliability of this quantification.

Conclusion

In conclusion we have shown that in the background of very low Cx43 levels, arrhythmogenic mice have a more severe reduction in Cx43 with global, but not local, heterogeneity of Cx43 expression. In regions with depletion of Cx43, Nav1.5 expression was also absent, which is presumably caused by direct interaction at the intercalated disc and leads to reduction of sodium current. This results in slowed and dispersed conduction which allows for the occurrence of ventricular arrhythmias.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1. Ventricular tachycardias

Panel A shows an epicardial electrogram (upper panel) of a sustained polymorphic tachycardia. The corresponding activation maps of the 3 numbered consecutive beats are presented below, showing irregular activation patterns. The upper panel in B shows an epicardial electrogram of a sustained monomorphic tachycardia, with comparable activation maps of 2 consecutive beats (lower panels). The yellow arrow in the schematics of the heart indicates fiber direction. Colors indicate areas activated within the same time interval and are similar for each map of one arrhythmia. Lines are isochronal lines at distances of 1ms. The red dotted lines correspond to $t=0$ in the activation maps. Panel C overviews the induction and type of arrhythmia of individual susceptible mice.

Figure 2. Activation maps

Typical epicardial activation patterns of LV (upper panels) and RV (lower panels) of Cx43^{Cre-ER(T)/fl} mice without (VT-) and with (VT+) arrhythmias during BCL (120ms, left panels) or premature stimulation (ERP+5ms, right panels). The pacing site is shown in the center of each activation map, resulting in anisotropic conduction. The yellow arrows in the schematics of the heart indicate fiber direction. Colors indicate areas activated within the same time interval and are similar for each map. Lines are isochronal lines at distances of 1ms.

Figure 3. Cx43 expression and heterogeneity

Typical pictures of Cx43 expression with corresponding N-cadherin staining of LV (left panels) and RV (right panels) of $Cx43^{Cre-ER(T)/fl}$ mice without (VT-) and with (VT+) arrhythmias. Whereas Cx43 expression is present throughout the hearts of VT- mice, regions completely devoid of Cx43 can be found in VT+ hearts (lower panels). Bars represent 50 μm.

Figure 4. Nav1.5 expression and fibrosis

Panel A shows typical pictures of Nav1.5 expression with corresponding N-cadherin staining of Cx43^{Cre-ER(T)/fl} mice without (VT-) and with (VT+) arrhythmias. Panel B shows a double labeling for both Cx43 and Nav1.5 of Cx43^{Cre-ER(T)/fl} mice without (VT-) and with (VT+) arrhythmias. The arrows in the pictures of VT+ mice indicate regions of co-localization of Cx43 and Nav1.5, whereas the asterisk points to a region of co-decrease. Typical pictures of Picrosirius Red staining of Cx43^{Cre-ER(T)/fl} mice without (VT-) and with (VT+) arrhythmias are shown in panel C. Bars represent 50 μm.

Figure 5. Decreased Cx43 expression and I_{Na} in isolated adult rat ventricular myocytes
 Panel A: Western blot for Cx43 in cells that were untreated (UNT), treated with an oligonucleotide targeting Cx43 expression (KD) or a non-targeting construct ϕ KD. Sodium current parameters were measured in the latter two cell populations, using standard single-electrode patch clamp methods. Panel B: Peak sodium current density was decreased in cells lacking Cx43 (black dots and lines; labeled “Cx43sil”) when compared to cells treated with the non-targeting construct (red lines and symbols; labeled “Cx43scr”). Panels C and D: Loss of Cx43 expression did not affect steady-state inactivation (C) or recovery from inactivation (D) kinetics.

Table 1

Induction-time, heart weight / body weight ratio, gender and electrophysiology

	VT- (n=10)	VT+ (n=13)
Induction-time (days)	13.8±0.6	13.5±0.3
HW/BW (%)	0.72±0.03	0.77±0.14
Male (%)	60	38
Arrhythmias (%)	0	100
LV CV _L (cm/s)	54.6±4.3	45.7±7.1
RV CV _L (cm/s)	47.2±4.2	40.9±6.2
LV CV _T (cm/s)	26.1±2.1	21.1±1.6
RV CV _T (cm/s)	27.7±1.6	22.5±2.8
LV AR (CV _L /CV _T)	2.11±0.08	2.23±0.22
RV AR (CV _L /CV _T)	1.72±0.13	1.82±0.15
LV CV Disp Index	1.41±0.15	1.90±0.19
RV CV Disp Index	1.41±0.17	2.31±0.36 *
LV ERP (ms)	70.0±10.1	75.6±7.3
RV ERP (ms)	47.0±3.4	46.1±4.1
QRS-duration (ms)	12.1±0.3	15.4±1.1 *
LV CV _L extra (cm/s)	41.0±4.4	34.4±6.7
RV CV _L extra (cm/s)	39.1±6.2	22.2±2.1 *
LV CV _T extra (cm/s)	22.0±2.5	17.1±2.1
RV CV _T extra (cm/s)	21.6±2.4	13.4±1.1 *
LV AR extra	1.88±0.06	1.92±0.20
RV AR extra	1.75±0.18	1.64±0.05

VT-, Cx43^{Cre-ER(T)/fl} mice without arrhythmias; VT+, Cx43^{Cre-ER(T)/fl} mice with arrhythmias; induction-time, time from start Tamoxifen-induction to Langendorff experiments; HW/BW, heart weight / body weight ratio; LV, left ventricle; RV, right ventricle; CV_L, longitudinal conduction velocity; CV_T, transversal conduction velocity; AR, anisotropic ratio; CV Disp, dispersion of conduction velocity; ERP, effective refractory period. Values are ± SEM.

* p<0.05 vs VT-.

Table 2

Tissue characteristics

	VT-	VT+
Cx43-exp LV (%)	0.25±0.06	0.05±0.04 *
Cx43-exp RV (%)	0.22±0.07	0.03±0.02 *
Cx43-het micro LV	0.39±0.02	0.38±0.03
Cx43-het micro RV	0.43±0.03	0.43±0.03
Cx43-het macro LV	0.22±0.03	0.34±0.03 *
Cx43-het macro RV	0.22±0.03	0.34±0.03 *
Nav1.5-exp (rank obs. #1)	15.48	30.86 **
Nav1.5-exp (rank obs. #2)	15.96	30.36 **
Nav1.5-exp (rank obs. #3)	15.70	30.64 **
Fibrosis LV (%)	0.48±0.12	0.51±0.13
Fibrosis RV (%)	0.54±0.09	0.49±0.04

Cx43-exp, Cx43 expression; Cx43-het, Cx43 heterogeneity; Nav 1.5-exp, Nav1.5 expression; rank obs. #1, average rank of observer 1; VT-, Cx43^{Cre-ER(T)/fl} mice without arrhythmias; VT+, Cx43^{Cre-ER(T)/fl} mice with arrhythmias. Values are ± SEM.

* p<0.05 vs VT-;

** p<0.005 vs VT-.