Reduced heterogeneous expression of Cx43 results in decreased Nav1.5 expression and reduced sodium current that accounts for arrhythmia vulnerability in conditional Cx43 knockout mice

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BACKGROUND Reduced expression of connexin43 (Cx43) and sodium channel (Nav1.5) and increased expression of collagen (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 knockout mice with and without inducible ventricular tachycardia (VT) were compared through electrophysiology and immunohistochemistry.

METHODS $Cx43^{CreER(T)/fl}$ mice were induced with tamoxifen and killed after 2 weeks. Epicardial activation mapping was performed on Langendorff-perfused hearts, and arrhythmia vulnerability was tested. Mice were divided into arrhythmogenic (VT+; n = 13) and nonarrhythmogenic (VT−; n = 10) animals, 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 than did VT− mice. Nav1.5-immunoreactive protein expression was lower in VT+ than in 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 was similar between the 2 groups. Premature stimulation resulted in a more severe conduction slowing in VT+ than in VT− hearts in the right ventricle. Separate patch-clamp experiments in isolated rat ventricular myocytes confirmed that the loss of Cx43 expression correlated with the 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

ABBREVIATIONS $\mathbf{CV_L}$ = longitudinal conduction velocity; $\mathbf{CV_T}$ = transversal conduction velocity; **Cx43** = connexin43; **ERP** = effective refractory period; $\mathbf{I_{Na}}$ = sodium current; **LV** = left ventricle; **RV** = right ventricle; **VT** = ventricular tachycardia

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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] is thought to impair the proper conduction of the electrical impulse, increasing the risk for fatal ventricular arrhythmias.[7] 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 in mice did not affect impulse conduction.[12,13] However, further reduced Cx43 levels by condi-

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tional deletion of the Cx43 gene to <5% resulted in a high vulnerability for arrhythmias owing to slowed and dispersed conduction.[13,14] It was then proposed,[13] as shown in a genetic model of heterogeneous Cx43 expression,[8] that the decreased and heterogeneous expression of Cx43 protein levels allowed for the occurrence of ventricular arrhythmias.

Despite the fact that genetic tools allow for specific downregulation 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 the 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 the 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, which results in slow and dispersed conduction. Moreover, given the interactions between Cx43 and the voltage-gated sodium channel complex,[19] 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 divided tamoxifen-induced $Cx43^{Cre\text{-}ER(T)/fl}$ mice into arrhythmogenic (VT+; VT = ventricular tachycardia) and nonarrhythmogenic (VT−) animals and compared conduction parameters and tissue characteristics. Our data show that VT+ mice have a more severe reduction in Cx43 protein levels than do 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 lower in VT+ than in VT− mice, whereas the collagen content was the same. Separate studies showed that the loss of Cx43 expression led to a decrease in the amplitude of I_{Na} in adult rat ventricular myocytes. Together, these 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 supplemental material. Brief descriptions are presented below.

Animals

$Cx43^{Cre\text{-}ER(T)/fl}$ (n = 17) mice were generated and injected with tamoxifen as described previously[13] and analyzed 13−15 days after the first injection. An extended analysis of previous data (n = 15) on similar tamoxifen-induced $Cx43^{Cre\text{-}ER(T)/fl}$ mice was included in this study.[13]

Preparation of the hearts and ventricular conduction

Electrocardiographic measurements and Langendorff experiments as well as data analysis are described in the online supplemental material.

Statistics

The statistical tests used are described in the online supplemental material.

Immunohistochemistry and histology

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

To quantify the heterogeneity of Cx43 expression, photographs were transformed into 8-bit white (Cx43) and black pictures and subdivided into 140 equal squares. The total intensity of Cx43 was determined for each square. Microheterogeneity was defined as the standard deviation of all squares divided by the mean in 1 photograph, whereas macroheterogeneity compared total intensities of all pictures of each ventricle of the heart. A detailed description can be found in the online supplemental material.

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 separately on every ranking.

Determination of I_{Na} properties in Cx43-knockdown cells

Cell isolation, transfection, and I_{Na} measurements were performed as described previously.[18,19] For a detailed description, see the online supplemental material.

Results

Arrhythmogeneity

We analyzed 32 tamoxifen-induced $Cx43^{Cre\text{-}ER(T)/fl}$ 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 mice, arrhythmias were induced by either premature stimulation or burst pacing (Figure 1C). The majority of arrhythmias (10 of 13) were sustained (>15 complexes followed stimulation). The other arrhythmias were nonsustained (~8 complexes followed stimulation; 1 heart) or premature beats (2 hearts). In the remaining 10 tamoxifen-induced $Cx43^{Cre\text{-}ER(T)/fl}$ mice, no arrhythmias could be induced.

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