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Cardiac-Specific Loss of N-Cadherin Leads to Alteration in Connexins With Conduction Slowing and Arrhythmogenesis

Jifen Li,* Vickas V. Patel,* Igor Kostetskii, Yanming Xiong, Antony F. Chu, Jason T. Jacobson, Cindy Yu, Gregory E. Morley, Jeffery D. Molkentin, Glenn L. Radice

Abstract—The remodeling of ventricular gap junctions, as defined by changes in size, distribution, or function, is a prominent feature of diseased myocardium. However, the regulation of assembly and maintenance of gap junctions remains poorly understood. To investigate N-cadherin function in the adult myocardium, we used a floxed N-cadherin gene in conjunction with a cardiac-specific tamoxifen-inducible Cre transgene. The mutant animals appeared active and healthy until their sudden death \approx 2 months after deleting N-cadherin from the heart. Electrophysiologic analysis revealed abnormal conduction in the ventricles of mutant animals, including diminished QRS complex amplitude consistent with loss of electrical coupling in the myocardium. A significant decrease in the gap junction proteins, connexin-43 and connexin-40, was observed in N-cadherin-depleted myocytes. Perturbation of connexin function resulted in decreased ventricular conduction velocity, as determined by optical mapping. Our data suggest that perturbation of the N-cadherin/catenin complex in heart disease may be an underlying cause, leading to the establishment of the arrhythmogenic substrate by destabilizing gap junctions at the cell surface. (*Circ Res.* 2005;97:474-481.)

Key Words: cell adhesion ■ arrhythmia ■ gap junctions ■ conditional knockout

Alterations in electrical coupling of ventricular myocytes play an important role in arrhythmogenesis in many forms of heart disease, including hypertrophy, ischemia, and dilated cardiomyopathies.¹ The molecular mechanisms underlying cellular uncoupling and the subsequent development of conduction abnormalities and arrhythmogenesis are poorly understood. Abnormal mechanical coupling through adherens junctions and desmosomes occurs in experimental animal hearts^{2,3} and diseased human myocardium^{4,5}; however, it is unclear whether these changes are responsible for the arrhythmogenesis observed in these patients.

The gap junction provides intercellular communication through electrical stimulus and small molecules that pass through a channel generated by a family of proteins called connexins.⁶ In heart disease, connexin 43 (Cx43), which is the primary connexin of ventricular myocardium, is often downregulated and preferentially lost from the intercalated disc.⁷⁻⁹ Cardiac-restricted deletion of the Cx43 gene in mice results in decreased ventricular conduction velocity, and these animals die suddenly because of spontaneous ventricular arrhythmias, demonstrating that loss of Cx43 is sufficient for development of the arrhythmogenic substrate.¹⁰⁻¹²

Classic cadherins, including E-, P-, and N-cadherin, constitute a family of cell surface glycoproteins that mediate

calcium-dependent adhesion.¹³ The classical cadherins are single pass transmembrane proteins with 5 extracellular domains, a transmembrane domain, and a cytoplasmic domain. Through their homophilic binding and adhesive specificities, cadherins are thought to play a critical role in tissue formation in the embryo and maintenance in the adult. A highly-conserved cytoplasmic domain that associates with a family of cytoplasmic proteins called catenins, (including α -catenin, β -catenin, γ -catenin (plakoglobin), and p120ctn), defines the classic cadherins. Formation of the cadherin-catenin complex is required for cadherin-mediated cell adhesion, and it is thought that α -catenin, which binds to the cadherin- β -catenin, or cadherin-plakoglobin complex, mediates linkage to the actin cytoskeleton.

Germline deletion of N-cadherin results in early embryonic lethality associated with multiple developmental abnormalities, including severe cardiovascular defects.¹⁴ In addition to its role in adherens junctions, N-cadherin is also involved in the formation and/or function of gap junctions.¹⁵ Observations of interactions between adult rat cardiomyocytes in culture demonstrated recruitment of N-cadherin to regions of cell-cell contact before the arrival of Cx43 to these newly established cell junctions, providing support that N-cadherin

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is important for gap junction assembly.^{16,17} In vitro studies with embryonic mouse¹⁸ and adult rat¹⁹ cardiomyocytes demonstrated that N-cadherin-mediated adhesion is critical for maintaining Cx43 at the plasma membrane.

Here we show that cardiac-restricted deletion of N-cadherin causes severe conduction defects and increased susceptibility for induced arrhythmogenesis associated with altered connexins. Both Cx40 and Cx43 were affected in the N-cadherin CKO animals, demonstrating that N-cadherin is responsible for stabilizing gap junctions in general, regardless of connexin subtype. This unique animal model demonstrates that N-cadherin is a critical determinant in the development of the arrhythmogenic substrate, which has implications for the development of sudden death in many forms of heart disease where mechanical coupling has been compromised, including hypertrophic, ischemic, and dilated cardiomyopathies.

Materials and Methods

Surface ECG and In Vivo Electrophysiology Studies

To induce Cre-mediated recombination, adult α MHC/MerCreMer, N-cad^{null/flox}, or N-cad^{flox/flox} mice were treated with Tamoxifen (TAM, Sigma) by intraperitoneal injection once a day for 5 consecutive days at a dosage of 2 mg/25g mice per day. Mice were analyzed in a mixed 129Sv/C57Bl/6J genetic background. Protocols for the in vivo mouse electrophysiology (EP) study have been described previously in detail.²⁰ A detailed description of the experimental methods is available in the online data supplement at <http://circres.ahajournals.org>.

Results

Sudden Death in Cardiac-Restricted N-Cadherin Mutant Mice

To overcome the requirement for N-cadherin-mediated adhesion during heart development, we used a N-cadherin conditional knockout mouse (N-cad^{flox/+}) and an inducible cardiac-specific Cre transgene.²¹ The N-cad^{flox/+} mice were bred with α myosin heavy chain (MHC)/MerCreMer transgenic mice to mediate cardiac-specific deletion of the N-cadherin gene after TAM administration.²² Homozygous N-cad^{flox/flox} mice were mated with α MHC/MerCreMer, N-cad^{null/+}, or N-cad^{flox/flox} mice as described previously.²¹ Six- to 10-week-old N-cad^{null/flox} or N-cad^{flox/flox} animals with the α MHC/MerCreMer transgene were administered TAM for 5 consecutive days. Controls included animals of the same genotype not given TAM and animals without the Cre transgene given TAM. In either case, no effect on N-cadherin expression was observed in the heart. As described previously,²¹ N-cadherin was lost from the intercalated disc \approx 3 weeks after TAM administration. The N-cadherin CKO animals exhibited normal activity and appeared healthy compared with their control littermates. However, the animals died suddenly between 1 to 2 months after TAM administration (Figure 1) from spontaneous ventricular tachyarrhythmias.²¹

ECG and Electrophysiologic Analysis of N-Cadherin CKO Mice

To better understand and characterize the contribution of N-cadherin to normal electrophysiologic function in the heart, we initially recorded surface ECGs from adult N-cadherin CKO and gender-matched Cre⁻ littermates 4 to 5 weeks after

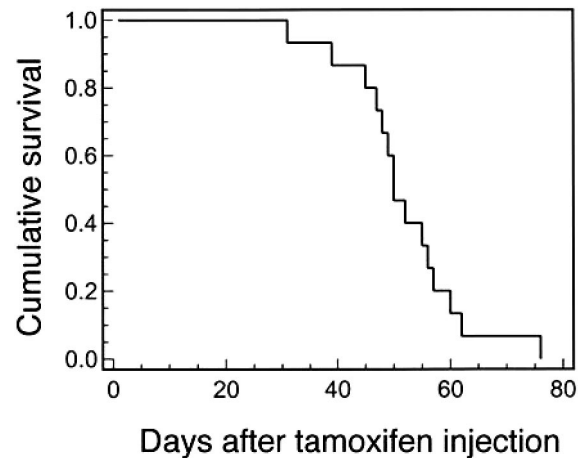


Figure 1. Kaplan–Meier survival curve after cardiac-specific depletion of N-cadherin. The mean time to sudden death was 50 days after tamoxifen administration (n=15).

TAM. The ECG analysis revealed marked abnormalities in N-cadherin CKO mice (Figure 2 and Table), which had longer PR intervals and QRS complex width. In addition, the QRS amplitude was lower and the p wave amplitude was larger with a wider duration in N-cadherin CKO mice, compared with their Cre⁻ littermates.

Invasive EP studies were subsequently performed in all mice that underwent surface ECG analysis to further characterize the contribution of N-cadherin to cardiac electrical function. Invasive analysis revealed that N-cadherin CKO mice had a significantly longer HV interval (representing impulse propagation through the His bundle and bundle branches), compared with their Cre⁻ littermates. However, the AH interval (representing impulse propagation through the atrium and the atrioventricular node), His-bundle dura-

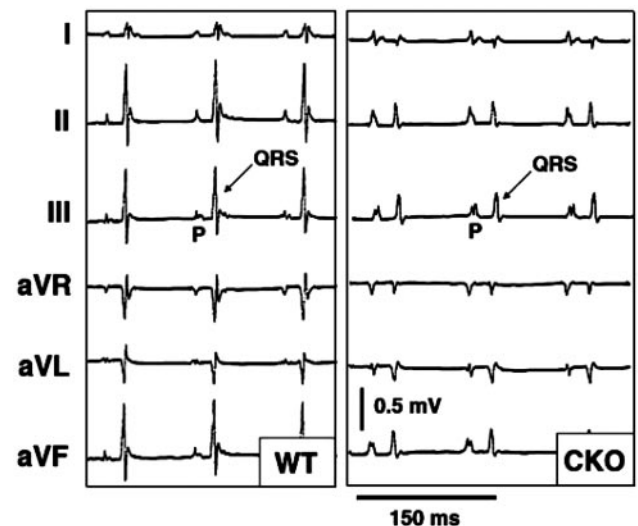


Figure 2. Cardiac-restricted loss of N-cadherin induces severe electrocardiographic changes. Shown from top to bottom are surface ECG leads I, II, III, aVR, aVL, and aVF from a wild-type (WT) and mutant (CKO) mouse. Compared with the same ECG leads from the WT mouse in panel A, the QRS complex is smaller and wider, whereas the p wave is taller and wider in the CKO mouse (B). In addition, the PQ interval is prolonged in the CKO animal compared with WT.

Baseline ECG Intervals and EP Analysis of N-Cad CKO Mice

	WT (n=8)	CKO (n=6)
After TAM Injection, d	34.0±4.2	33.3±4.4
Age, d	87.5±10.4	83.8±11.4
Weight, g	26.3±2.5	22.7±5.3
SCL, ms	190±25.5	183±17.5
HR, bpm	321±42.7	330±30.8
PR, ms	42.2±3.9	50.8±4.4*
QRS, ms	12.7±1.6	16.4±2.6*
QT, ms	26.8±2.4	23.9±3.6
QT _m , ms	19.5±2.4	17.9±2.6
P wave duration, ms	12.1±1.7	15.2±2.4*
P wave amp, mV	0.13±0.05	0.19±0.08*
QRS amp, mV	0.55±0.28	0.21±0.12*
PA, ms	8.1±1.7	11.3±2.1*
AH, ms	37.8±6.9	36.2±4.2
H _d , ms	5.5±1.2	5.4±0.9
HV, ms	13.2±1.0	16.4±0.9*
AVI, ms	45.8±4.6	52.8±3.7*
SNRT ₁₅₀ , ms	210±24.9	219±47.0
AVERP ₁₅₀ , ms	78.0±10.4	93.3±2.9
AVERP ₁₂₀ , ms	75.8±13.2	82.0±18.9
AWWBCL, ms	107±13.2	113±16.4
AV 2:1, ms	87.9±14.1	92.5±10.8
AERP ₁₅₀ , ms	42.0±4.1	46.7±2.9
VERP ₁₅₀ , ms	35.0±5.0	37.5±4.7

**P*<0.01 compared to control.

SCL indicates sinus cycle length; HR, heart rate; PR, PR interval duration; QRS, QRS complex width; QT, QT interval duration; QT_m, corrected QT interval duration; PA, intra-atrial conduction time; AH, atriohisian interval; H_d, his-bundle duration; HV, hisioventricular interval; AVI, atrioventricular interval; SNRT₁₅₀, sinus node recovery time after a conditioning train at 150 ms; AERP₁₅₀, atrial effective refractory period (ERP) after a drive train at 150 ms; AVERP₁₅₀, atrioventricular ERP after a drive train at 150 ms; AVERP₁₂₀, atrioventricular ERP after a drive train at 120 ms; AWWBCL, atrioventricular Wenckebach block cycle length; AV 2:1, atrioventricular 2:1 block cycle length; VERP₁₅₀, ventricular ERP after a drive train at 150 ms.

tion, sinus node recovery time, atrioventricular nodal conduction, and refractory periods in the atrium and ventricle were not significantly different (Table).

Ventricular programmed electrical stimulation with up to 3 extrastimuli induced 5 episodes of ventricular tachycardia in 3 of 6 of the N-cadherin CKO mice, which lasted 38.7±20.6 s (range: 12.1 to 77.3 s), whereas none was induced in any of the Cre⁻ littermates (*P*<0.01). Burst ventricular pacing at a cycle length of 50 ms induced 6 episodes of ventricular tachycardia in 4 of 6 N-cadherin CKO mice, which lasted 107.2±39.0 s (range: 22.8 to 198.5 s), whereas only one Cre⁻ littermate had a single nonsustained episode of ventricular tachycardia with burst pacing, which lasted 0.33 s (*P*<0.01, Figure 3).

Atrial programmed electrical stimulation with up to 3 extrastimuli induced 6 episodes of atrial arrhythmia in 4 of 6 of the N-cadherin CKO mice, which lasted 742±290 ms (range: 243 to 1057 ms), whereas none was induced in any of

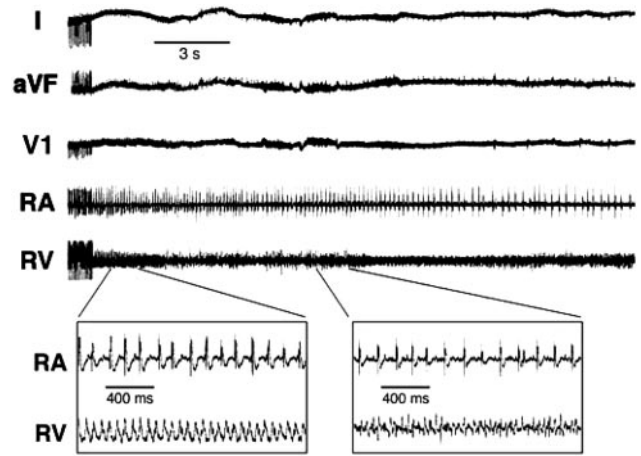


Figure 3. Ventricular arrhythmia induced by burst pacing in a N-cadherin CKO mouse. Shown from top to bottom are surface ECG leads I, aVF, and V1, along with intracardiac recordings from the right atrium (RA) and right ventricle (RV). Burst pacing delivered at a cycle length of 50 ms to the RV is shown at the left of the figure, induced monomorphic ventricular tachycardia for several seconds with AV dissociation (left enlargement). The ventricular tachycardia quickly degenerated into ventricular fibrillation (right enlargement), which persisted for several minutes.

the Cre⁻ littermates (*P*<0.01; Figure 4). Burst atrial pacing at a cycle length of 50 ms induced 7 episodes of atrial tachycardia in all 6 N-cadherin CKO mice, which lasted 982±396 ms (range: 430 to 1368 ms), whereas only 1 of 8 Cre⁻ littermates had 2 episodes of atrial arrhythmia with burst pacing that lasted 480 ms and 586 ms, respectively (*P*<0.01). Atrial electrical stimulation did not induce ventricular arrhythmias in any animals studied.

Disassembly of Gap Junctions in N-Cadherin-Depleted Hearts

We speculated that altered gap junctions caused the abnormal conduction properties leading to lethal cardiac arrhythmias. Therefore, we examined the expression of Cx43, the predominant gap junction protein in the ventricular myocardium, and

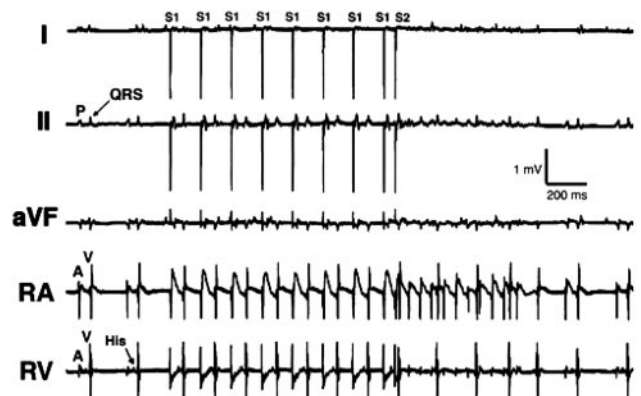


Figure 4. Atrial arrhythmia induced by programmed stimulation in a N-cadherin CKO mouse. Shown from top to bottom are surface ECG leads I, II, and aVF, along with intracardiac recordings from the right atrium (RA) and right ventricle (RV). A drive-train delivered at 150 ms (S1), coupled to a single premature stimulus at 50 ms (S2) in the right atrium, induced nonsustained atrial fibrillation with an irregular ventricular response.

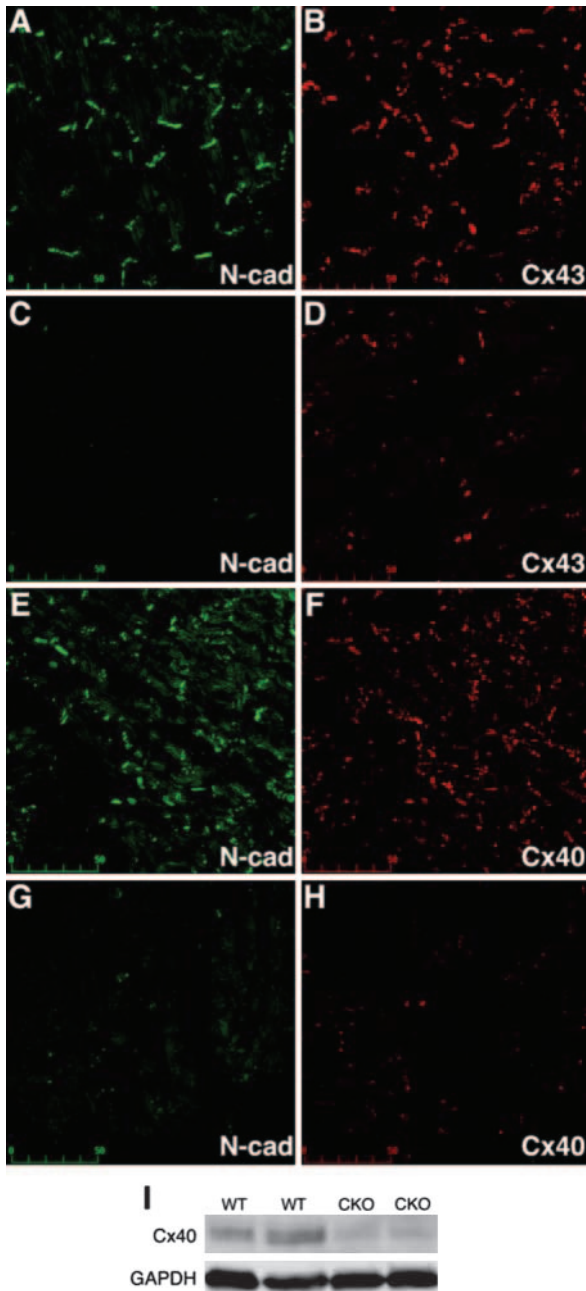


Figure 5. Expression of connexins in N-cadherin CKO hearts. Hearts from wild-type (A, B, E, and F) and N-cadherin CKO (C, D, G, and H) animals 7 weeks after Tam were coimmunostained for N-cadherin (A and C) and Cx43 (B and D) in the ventricle, and N-cadherin (E and G), and Cx40 (F and H) in the atrium. N-cadherin was lost from the intercalated disc in the CKO heart, whereas Cx43 (D) and Cx40 (H) were significantly decreased in the ventricular and atrial myocardium, respectively. Western blot of Cx40 in atrial lysates from wild-type (WT) and N-cadherin mutant (CKO) mice. GAPDH signal shows relative loading of samples. Bar=50 μ m.

Cx40 in the N-cadherin-depleted hearts. N-cadherin CKO hearts were doublestained with N-cadherin and Cx43 or Cx40 after TAM administration. Depletion of N-cadherin in the intercalated disc resulted in a significant decrease in both Cx43 and Cx40 in ventricular and atrial myocardium, respectively (Figure 5). Both Cx40 and Cx43 (see online data supplement) were decreased in atrial myocardium in the

absence of N-cadherin. Quantification of the Cx43 immunofluorescence in the ventricular myocardium 25 days after TAM showed reduced aggregate number (45%), size (50%), intensity (20%), and percent area occupied by Cx43 immunofluorescence signal (22%) in the CKO, compared with WT control ($P<0.001$; $n=4$), thus demonstrating heterogeneous Cx43 expression. Western blot analysis of atrial lysates confirmed a reduction in Cx40 in the N-cadherin CKO compared with wild-type hearts (Figure 5I). We could not detect Cx45 in wild-type or N-cadherin CKO hearts by immunoblotting, consistent with its restricted expression in the heart, compared with Cx43 and Cx40. The cytoskeletal adaptor protein, zona occluden-1 (ZO-1), which binds to the carboxy tail of Cx43²³ and colocalizes with N-cadherin,²⁴ was lost from the intercalated disc but maintained its lateral border localization (see online data supplement).

To follow the time course of N-cadherin and Cx43 turnover, quantitative Western blot analysis was performed on heart lysates at 6, 15, 25, and 41 days after TAM administration (Figure 6). Two representative samples are shown for each time point ($n=6$). N-cadherin was significantly decreased by day 6, reaching 20% of endogenous levels by day 25. Connexin 43 is phosphorylated when it forms an active gap junctional complex, or channel, at the cell surface.²⁵ In contrast, it is dephosphorylated during trafficking/endocytosis in the cytoplasm. Total Cx43 was examined using an antibody that recognizes both phosphorylated and nonphosphorylated Cx43 (Figure 6). Connexin 43 decreased significantly by day 15, reaching 40% of endogenous levels by day 41. There was also a shift from the slower migrated phosphorylated species to the faster migrating dephosphorylated species, coincident with depletion of N-cadherin (Figure 6). To directly examine the dephosphorylation status of the protein, an antibody was used that recognizes Cx43 only when the serine at residue 368 is unphosphorylated.²⁶ Interestingly, a significant increase (2.5-fold) in the dephosphorylated species was observed at day 6, consistent with increased turnover of the protein immediately after loss of N-cadherin (Figure 6B).

To determine whether Cx43 expression was affected at the mRNA level, real-time PCR analysis was performed on hearts at 6, 15, 25, and 41 days after TAM administration (Figure 6C). Connexin 43 mRNA levels showed a modest increase (2.0-fold) after depletion of N-cadherin, indicating posttranscriptional regulation was responsible for the downregulation of Cx43.

Conduction Slowing in N-Cadherin CKO Hearts

To directly examine the consequences of loss of N-cadherin on ventricular conduction, we optically mapped paced electrical activity in the hearts of N-cadherin CKO mice and littermate controls using a voltage-sensitive dye.²⁷ Compared with control mice, conduction velocity (CV) was significantly reduced, as visualized by optical mapping in the N-cadherin CKO hearts (Figure 7). As a result, the anisotropic ratio (CV_{max}/CV_{min}) was increased in the CKO LV (1.85 in control versus 2.21 in CKO, $P<0.05$, $n=6$). The slowing of ventricular conduction and increased anisotropy was similar to that

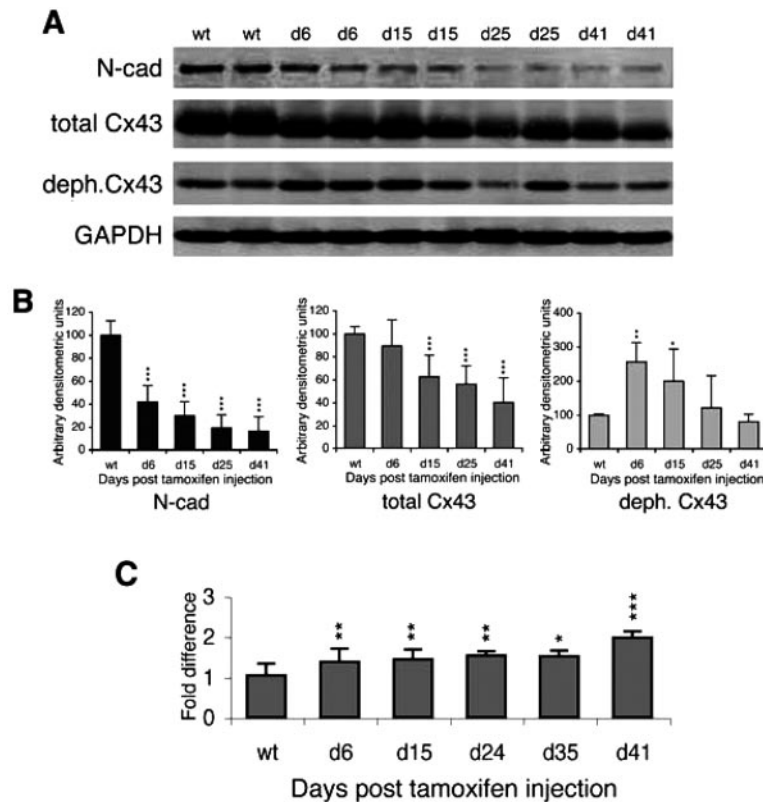


Figure 6. Time course of N-cadherin and Cx43 turnover in N-cadherin CKO hearts. A, Quantitative Western blot analysis was performed on heart lysates from wild-type (WT) and N-cadherin CKO mice 6 days (d6), 15 days (d15), 25 days (d25), and 41 days (d41) after Tam. Blots were probed for N-cadherin, total Cx43, and dephosphorylated Cx43 (ser 368). B, Six independent animals were examined for each time point and compared with WT. GAPDH signal was used to normalize for loading differences between lanes. C, Quantification of Cx43 mRNA expression by real time PCR was performed at same time points as the Western analysis. *** $P < 0.001$; ** $P < 0.01$; * $P < 0.05$.

observed in Cx43 CKO mice,¹¹ providing further evidence that N-cadherin affects gap junction function.

Discussion

We have shown previously that postnatal cardiac-restricted deletion of N-cadherin leads to complete dissolution of the intercalated disc structure with a modestly dilated cardiomyopathy.²¹ We noted sudden death in a majority of animals ≈ 6 to 8 weeks after inducing deletion of N-cadherin in the heart that was correlated with the onset of spontaneous ventricular arrhythmias. The mechanism by which depleting N-cadherin creates a substrate for ventricular arrhythmogenesis and sudden death in this model is not entirely clear, but is likely related to loss of functional gap junctions.

In line with the role of connexins contributing to the arrhythmogenic substrate, the more comprehensive EP analysis presented in the current study reveals striking similarities between the phenotypes of Cx43 CKO models generated by other groups^{10,11} and our N-cadherin CKO mice. Surface ECG analysis shows that the QRS amplitude is significantly reduced in N-cadherin CKO mice, similar to that seen in Cx43 CKO mice.^{10,28} Invasive EP analysis reveals that the ventricular effective refractory periods are not significantly affected, compared with control littermates in either N-cadherin CKO or Cx43 CKO mice, and both cardiac-restricted knockout models have an increased propensity for spontaneous and induced ventricular arrhythmias.^{12,28} Also, the remaining gap junctions are heterogeneously scattered throughout the myocardium in both models. Finally, epicardial conduction velocity is similarly reduced as the result of cardiac-restricted deletion of Cx43^{11,12} or N-cadherin. It is the

last 2 characteristics, slowed conduction velocity and gap junction remodeling, that most likely contribute to the increased arrhythmogenicity in our N-cadherin CKO mice.

The reduced and heterogeneous distribution of Cx43 within the ventricle of N-cadherin CKO mice is likely to contribute to electrical uncoupling between myocytes and forms the nonuniformities required to initiate breaks within a propagating wave front and/or unidirectional block.^{29,30} Tachycardia wavelength (λ) is a function of conduction velocity (CV) and refractory period (ERP) where $\lambda = CV \times ERP$,³¹ so slowed conduction velocity (which actually reflects reduced functional connexin expression) will decrease the tachycardia wavelength and allow more space for the initiated wave breaks to curl up and form rotors. In our N-cadherin CKO mice, the tachycardia wavelength (51 cm/s \times 0.0375 s = 19 mm) is indeed smaller compared with control littermates (92 cm/s \times 0.035 s = 32 mm). Although the smaller wavelength probably contributes an increased propensity for arrhythmogenesis in the absence of N-cadherin, the $\approx 50\%$ reduction in conduction velocity is not likely to significantly increase arrhythmogenicity by itself.^{12,28} In order for reentrant arrhythmogenesis to occur, the propagating wave front must form wave breaks, which then have enough space to curl up on themselves and form rotors.³² In this regard, Danik et al²⁸ have demonstrated that heterogeneous loss of connexin in Cx43 CKO mice is directly correlated with an increased propensity for arrhythmogenesis. We see a similar heterogeneous reduction of Cx43 in the N-cadherin-depleted heart, and this is likely to introduce electrical nonuniformities which initiate breaks within a propagating wavefront, and initiate rotors.²⁸⁻³⁰

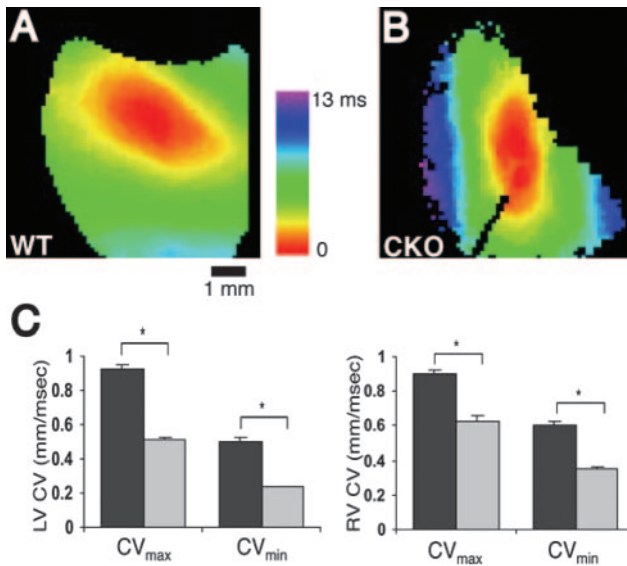


Figure 7. Optical-mapping studies in N-cadherin CKO mice. A, Left ventricular free wall of a control littermate heart showing the expected smooth epicardial activation pattern from the site of pacing at the lateral wall and spreading toward the ventricular septum. B, Representative epicardial activation pattern of an age-matched N-cadherin CKO mouse heart paced in the same fashion as the control heart. C, Comparison of CV_{max} and CV_{min} in left ventricle (LV) and right ventricle (RV) for the control (black bar) and N-cadherin CKO (gray bar) mice that were successfully paced ($P < 0.05$; $n = 6$ in each group). CV_{max} in control hearts was 0.92 mm/ms in LV and 0.90 mm/ms in RV, and CV_{min} was 0.51 mm/ms in LV and 0.60 mm/ms in RV. CV_{max} in N-cadherin CKO hearts was 0.51 mm/ms in LV and 0.63 mm/ms in RV, and CV_{min} was 0.23 mm/ms in LV and 0.35 mm/ms in RV.

Increased anisotropic conduction is another factor that may contribute to the propensity for arrhythmogenesis in the absence of N-cadherin. In the normal heart, there is usually greater cell–cell coupling and electrical conductance in a direction parallel to the long axis of the cardiac fiber (longitudinal), compared with a perpendicular (transverse) direction along the short axis of the fiber. These different conductive properties of the heart depend on the orientation of the fibers and produce conduction anisotropy where action potentials propagate faster in the longitudinal direction compared with the transverse direction. In our model, deletion of N-cadherin results in an increase in the anisotropy ratio (AR), where transverse conduction is more greatly reduced than is longitudinal conduction, with an increase in the AR. Although the increase in AR in the N-cadherin CKO animals is modest, it is significant and is probably the result of heterogeneous cellular uncoupling which contributes preferentially to propagation in the longitudinal direction and conduction slowing in the transverse direction. These heterogeneities in conduction and coupling are likely to promote arrhythmogenesis of multiple etiologies,³³ including that of the leading circle type, anisotropic reentry,³⁴ or even focal triggers. Therefore, loss of functional gap junctions induces partial cellular uncoupling, slowed conduction velocity with a reduction in tachycardia wavelength, and increased tissue anisotropy, all of which probably contribute to potentiate arrhythmogenesis when N-cadherin is deleted from the heart.

Analogous to our finding of reduced Cx43 in ventricular myocardium with an increased propensity for ventricular tachyarrhythmias in N-cadherin CKO mice, these animals also have an increased propensity for induced atrial arrhythmias with a parallel reduction and heterogeneous pattern of Cx40 and Cx43 within the atrial myocardium. In the rodent heart, Cx40 is expressed primarily in the atrium and central conduction system.³⁵ Knockout of Cx40 in mice induces conduction disturbances and predisposes them to atrial arrhythmias.^{36,37} Although we do not have measurements of conduction velocity from the atrium of N-cadherin CKO mice, because the level of reduction and heterogeneous distribution of Cx40 and Cx43 in the atrium mirrors that of Cx43 in the ventricle of N-cadherin CKO mice, it is likely that conduction velocity is similarly slowed in the atrium. Therefore, the mechanism underlying the propensity for induced atrial arrhythmogenesis in this model is likely similar to that for the ventricular arrhythmias, and related to the loss of the corresponding major connexin protein in their respective cardiac chamber.

Surface ECG analysis of N-cadherin CKO mice revealed that they had significantly widened QRS complex duration with lower overall amplitude. The relative increase in QRS duration seen in the Cx43 null model developed by Eckardt and colleagues¹⁰ is very similar to the increase we observed in our N-cadherin CKO model. However, in our N-cadherin CKO mice, Cx43 is only reduced to $\approx 40\%$ of wild-type levels, compared with a 95% reduction in the Cx43 CKO models.^{10,11} It is difficult to ascribe the QRS widening we see to just a 40% reduction in total Cx43 protein. In this regard, we believe that loss of N-cadherin causes a significant loss in functional gap junctions, as illustrated by the rapid increase in nonfunctional dephosphorylated Cx43 isoform 6 days after TAM treatment. The progressive dephosphorylation of Cx43 is associated with electrical uncoupling in other animal models^{38,39} consistent with our findings. Therefore, the total Cx43 remaining in the N-cadherin CKO heart is not meaningful if it cannot be assembled into functional gap junctions at the intercalated disc. It is likely that the reduction in QRS amplitude, and increase in QRS width, is related to the loss of cell–cell coupling in the absence of N-cadherin, consistent with observations made in cultured cardiomyocytes.⁴⁰

N-cadherin CKO mice show no abnormalities in sinus node function, with unaltered sinus rates and sinus node recovery, and have normal atrioventricular conduction with unaltered atriohisian (AH) conduction. The PR interval reflects myocardial conduction through the atrium, atrioventricular node, and the infranodal conduction system.⁴¹ Although N-cadherin CKO mice do have PR interval prolongation, this appears to be the result of delayed intra-atrial conduction (P wave prolongation and increase PA interval likely attributable to atrial enlargement), and slowed infrahisian conduction, as suggested by HV interval prolongation without actual atrioventricular nodal conduction defects.

A mutation in the cadherin binding partner, plakoglobin, is associated with the rare recessive disorder known as Naxos disease.⁴² Plakoglobin can bind to the cytoplasmic domain of classical and desmosomal cadherins, thus mediating linkage to the actin cytoskeleton and intermediate filaments, respec-

tively. Patients with Naxos disease have arrhythmogenic right ventricular cardiomyopathy associated with a high incidence of sudden cardiac death. The patients are initially identified with skin abnormalities, including nonepidermolytic palmoplantar keratoderma, and wooly hair. It was recently demonstrated that Cx43 levels are significantly decreased in the hearts of Naxos patients, including a young patient who did not exhibit clinical or pathological features of structural heart disease.⁴³ Interestingly, we observed a significant decrease in plakoglobin after deleting N-cadherin; in comparison, desmoplakin, another desmosomal linker protein, did not change significantly in total heart lysates.²¹ In an adult rat cardiomyocyte model, there was a progressive increase in the association of plakoglobin with the cadherin/catenin complex as intercalated disc-like structures formed in culture.⁴⁴ This raises an interesting question as to whether gap junctions are stabilized through N-cadherin/ β -catenin or N-cadherin/plakoglobin complexes in the heart.

In conclusion, we identified a new mechanism for cardiac arrhythmia resulting from loss of the cell adhesion molecule, N-cadherin, which is required to maintain functional gap junction complexes at the plasma membrane. Furthermore, our model suggests that structural remodeling of intercellular connections precedes electrical remodeling in the working myocardium. Hence, our results indicate that N-cadherin is important for the preservation of normal electrical function in the adult heart. Although several cytoskeletal proteins, or adapters (such as ankyrin-B), are important for maintaining anchorage of the contractile apparatus or ion channel activity, none of these proteins directly contributes to both mechanical and electrical coupling between cardiomyocytes like N-cadherin. It is likely that electrical uncoupling, caused by loss of functional gap junctions, is responsible for establishing a heterogeneous substrate to support arrhythmogenesis in the absence of N-cadherin. At this time no disease states are known, because of mutations of N-cadherin in humans, but the mechanism of arrhythmogenesis when N-cadherin function is perturbed has important implications for patients with heart disease, which may lead to better screening methods to identify persons at risk of sudden death, and possibly to novel therapies.

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