Accelerated Onset and Increased Incidence of Ventricular Arrhythmias Induced by Ischemia in Cx43-Deficient Mice

Deborah L. Lerner, MD; Kathryn A. Yamada, PhD; Richard B. Schuessler, PhD; Jeffrey E. Saffitz, MD, PhD

Background—Myocardial ischemia causes profound changes in both active membrane currents and passive electrical properties. Because these complex changes develop and progress concomitantly, it has not been possible to elucidate the relative contributions of any one component to arrhythmogenesis induced by acute ischemia. Cx43+/− mice express 50% of the normal level of connexin43 (Cx43), the major ventricular electrical coupling protein, but are otherwise identical to wild-type (Cx43+/+) mice. Comparison of arrhythmogenesis in Cx43+/− and +/+ mice can provide insights into the role of changes in electrical coupling as an independent variable in the complex setting of acute ischemia.

Methods and Results—Acute ischemia was induced in isolated perfused mouse hearts by occlusion of the left anterior descending coronary artery. Spontaneous ventricular tachyarrhythmias (VT) occurred in more than twice as many Cx43+/− hearts than Cx43+/+ hearts. VT was induced in nearly 3 times as many Cx43+/− hearts. Multiple runs and prolonged runs of spontaneous VT were more frequent in Cx43+/− hearts. Onset of the first run of VT occurred significantly earlier in Cx43+/− hearts. Premature ventricular beats were also more frequent in Cx43+/− hearts. The size of the hypoperfused region was equivalent in both groups.

Conclusions—Reduced expression of Cx43 accelerates the onset and increases the incidence, frequency, and duration of ventricular tachyarrhythmias after coronary artery occlusion. Thus diminished electrical coupling per se plays a critical role in arrhythmogenesis induced by acute ischemia. (Circulation. 2000;101:547-552.)

Key Words: arrhythmia • conduction • electrophysiology • ischemia

Sudden cardiac death has been described as an “electrical accident” that arises from complex, highly variable interactions between anatomic/functional myocardial substrates, transient initiating events, and cellular/tissue arrhythmia mechanisms.1 For example, malignant ventricular arrhythmias arising in the setting of acute myocardial ischemia are fundamentally related to marked reductions in tissue pH, increases in interstitial potassium and intracellular calcium levels, and neurohumoral changes, all of which interact in a complex, integrative pathophysiological milieu to slow conduction, alter excitability and refractoriness, promote electrical uncoupling, and generate spontaneous electrical activity.1-3 Increasingly sophisticated computer models of cardiac myocyte electrophysiology and metabolism have been useful in sorting out the contributions of alterations in specific proteins, channels, currents, or pathophysiological processes in arrhythmogenesis.4,5 However, gaining insights into the specific roles of individual determinants of arrhythmias in experimental systems involving real cardiac cells and tissues has not been possible previously.

Recent development of mouse models in which expression of specific gene products has been manipulated genetically provides an opportunity to define the roles of specific proteins in complex pathophysiological processes such as arrhythmogenesis induced by acute ischemia. We have previously studied mice6,7 in which expression of connexin43 (Cx43), the major ventricular gap junction protein, has been knocked out by homologous recombination.8 In contrast to Cx43-null animals, which die at birth, heterozygotes (Cx43+/−) survive and breed. Active membrane properties appear to be identical in Cx43-deficient (Cx43+/−) and wild-type (Cx43+/+) ventricular myocytes.6 However, Cx43+/− mice exhibit slow ventricular conduction that appears to be attributable solely to diminished coupling caused by expression of only 50% of the wild-type level of Cx43.6,7

We characterized ventricular tachyarrhythmias (VT) in isolated perfused mouse hearts subjected to left anterior descending (LAD) coronary occlusion. Comparison of wild-type and Cx43-deficient mice under identical conditions provided a means to investigate electrical coupling as an independent variable in arrhythmogenesis in the complex setting of acute ischemia. Using this approach, we found that
diminished electrical coupling per se accelerates the onset and increases the incidence of tachyarrhythmias induced by acute ischemia.

Methods

Cx43-Deficient and Wild-Type Mice

Hearts were isolated from 12- to 24-week-old mice produced from founders (B6;129-Gja1tm1Kdr) originally purchased from the Jackson Laboratories (Bar Harbor, Me) and maintained in an inbred background. Mice were housed in barrier facilities under strict veterinary supervision. Genotypes were determined by polymerase chain reaction as previously described, modified only by changing the primer sequences used. Hearts were perfused by retrograde aortic flow with oxygenated buffer at 37°C. Flow rates were adjusted to maintain constant perfusion pressures of 45 to 50 mm Hg. Hearts were simultaneously superfused in a bath containing oxygenated buffer at 37°C to maintain constant temperature.

All hearts were perfused with oxygenated buffer for an initial 20-minute equilibration period. Any heart that demonstrated contractile dysfunction or other evidence of injury was discarded. Two groups of hearts were studied after the 20-minute stabilization interval. One group of 5 Cx43−/− and 5 Cx43+/+ hearts was perfused with oxygenated buffer for an additional 60 minutes and served as controls for the hearts subjected to coronary occlusion. Another group of 16 Cx43−/− and 16 Cx43+/+ hearts was subjected to acute regional ischemia of the apical and anterolateral left ventricle for 60 minutes by ligating the LAD immediately distal to its origin with 8-0 Prolene suture. Perfusion flow rates were adjusted after coronary occlusion to maintain perfusion pressures of 45 to 50 mm Hg. Studies of Cx43+/+ hearts were performed by delivering a train of 8 beats at a basic cycle length of 130 ms (S1 s) followed by delivery of a single premature beat (S2). S2 was decremented by 5 ms in multiple runs to determine the effective refractory period. Attempts were made to induce ventricular tachycardia with single extrastimuli down to a coupling interval of 20 ms and with ventricular burst pacing (18 S1s) at intervals of 20 to 80 ms until 1:1 capture was achieved. This protocol was repeated 3 times at each cycle length. Throughout these procedures, hearts were continuously monitored for premature ventricular beats (PVBs) and VT. Induced or spontaneously occurring PVBs and VT were recorded and analyzed. A run of VT was defined as 10 or more beats with a cycle length <100 ms. The number, time of onset, and duration of these events were tabulated for each heart. Both spontaneous and induced arrhythmias were tabulated to maximize opportunities to compare arrhythmogenensis in Cx43−/− and +/+ hearts.

Statistical Analyses

All data are expressed as mean±SD. Comparisons of the incidence, frequency, and duration of VT in Cx43−/− and +/+ hearts were made with the Pearson χ² test. Continuous data such as cycle length and effective refractory period were compared with the use of ANOVA. Multiple comparisons between groups were made with Fisher’s least significant differences method. The time of onset of the first PVB or the first run of VT was analyzed by the Kaplan-Meier method, and comparisons between Cx43−/− and +/+ hearts were made with the Mantel log-rank test. A value of P<0.05 was considered significant.

Results

Electrophysiological Stability of the Isolated Perfused Mouse Heart

All control hearts (not subjected to coronary occlusion) remained in sinus rhythm and contracted vigorously throughout the 60-minute perfusion interval. Spontaneous cycle lengths were equivalent in control hearts of both genotypes and increased by ∼50 ms over the 60-minute interval (Table 1). Effective refractory periods did not change during perfusion. The effective refractory period was modestly lower in Cx43−/− compared with Cx43+/+ hearts, but this difference achieved statistical significance only at the initial

<table>
<thead>
<tr>
<th>TABLE 1. Cycle Length and Effective Refractory Period in Control Hearts</th>
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<tr>
<td>Cx43+/− (n=5)</td>
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<td>----------------</td>
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<tr>
<td>At onset of perfusion*</td>
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<tr>
<td>Cycle length, ms</td>
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<td>Effective refractory period, ms</td>
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<td>After 60 minutes of perfusion*</td>
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<tr>
<td>Cycle length, ms</td>
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<td>Effective refractory period, ms</td>
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*After the 20-minute stabilization period.
†P<0.01 vs Cx43+/++.
0-minute time point. A single PVB was recorded in only 1 of 5 Cx43\(^{1/2}\) hearts during the entire 60-minute normoxic perfusion period. Of the 5 Cx43\(^{1/2}\) hearts, 1 developed 1 PVB, 1 developed 2 PVBs, and 1 exhibited 20 premature beats during the 60-minute perfusion period. No runs of spontaneous or pacing-induced VT occurred in any control Cx43\(^{1/2}\) or \(^{1/1}\) hearts. These observations indicate that the isolated perfused mouse heart preparation is electrically stable during oxygenated perfusion for 60 minutes.

**Electrophysiological Changes in Cx43\(^{1/2}\) and Cx43\(^{1/1}\) Hearts Induced by Acute Regional Ischemia**

The LAD was occluded in 16 Cx43\(^{1/2}\) and 16 Cx43\(^{1/1}\) hearts, producing acute ischemia of the apical and anterolateral left ventricle. Spontaneous cycle lengths and effective refractory periods measured immediately before coronary artery occlusion in these hearts were similar to those shown in Table 1 for control hearts at the 0-minute time point. During the subsequent 60 minutes of regional hypoperfusion, spontaneous cycle lengths became prolonged and the effective refractory periods became shorter, but no significant differences in these values between Cx43\(^{1/2}\) and Cx43\(^{1/1}\) hearts occurred during the 60-minute interval of ischemia. Cycle lengths and effective refractory periods could not be determined in every heart at all time points because some exhibited persistent arrhythmias.

VT occurred in both Cx43\(^{1/2}\) and \(^{1/1}\) ischemic hearts. Figure 1 shows pairs of simultaneously recorded right atrial and ventricular electrograms and illustrates examples of the types of arrhythmias observed after coronary artery occlusion. Figure 1A demonstrates PVBs and a brief run of VT that initiated and terminated spontaneously. Figure 1B shows a polymorphic tachycardia with variable cycle lengths (53 to 74 ms) and electrogram morphology. Figure 1C shows a monomorphic tachycardia with uniform cycle lengths (55 to 60 ms) and electrogram morphology. VT cycle lengths were equivalent in the Cx43\(^{1/2}\) and \(^{1/1}\) hearts with arrhythmias (62\(\pm\)12 and 59\(\pm\)7 ms, respectively, \(P>0.5\)). The great majority of tachycardias terminated spontaneously. In a few cases (<5%), the tachycardia was terminated by the introduction of an extrastimulus.

**Ventricular Tachyarrhythmias in Cx43\(^{1/2}\) and Cx43\(^{1/1}\) Hearts**

Figure 2 shows the incidence of VT in Cx43\(^{1/2}\) and Cx43\(^{1/1}\) hearts after LAD occlusion. Of the 16 hearts in each group, 12 Cx43\(^{1/2}\) and 7 Cx43\(^{1/1}\) hearts had at least 1 run of either spontaneous or pacing-induced VT (Figure 2A). In addition, 11 Cx43\(^{1/2}\) hearts had multiple (>1) runs of VT compared with only 5 Cx43\(^{1/1}\) hearts (\(P<0.05\)). Four Cx43\(^{1/2}\) hearts exhibited nearly continuous bursts of VT with minimal intervening sinus rhythm during the final 30 minutes of ischemia compared with none of the wild-type

![Figure 1](image-url)  
*Figure 1. Examples of arrhythmias in isolated perfused mouse hearts after coronary artery occlusion. Each panel shows pairs of right atrial (Atr) and ventricular (Ven) epicardial electrograms recorded simultaneously. A, Premature ventricular beats (arrows) and a short (~1 second) run of VT that initiated and terminated spontaneously. The absence of a ventricular beat after the second atrial beat suggests a long refractory period after the PVB. B, Run of spontaneous polymorphic VT (cycle lengths from 53 to 74 ms). C, Run of spontaneous monomorphic VT (cycle lengths from 55 to 60 ms). Bar=100 ms.*

![Figure 2](image-url)  
*Figure 2. Incidence, frequency, and duration of spontaneous and/or pacing-induced VT after coronary artery occlusion in Cx43\(^{1/2}\) and \(^{1/1}\) hearts (n=16 for each group). * \(P<0.05\), † \(P<0.01\) by \(\chi^2\) analysis.*
hearts. Twelve of 16 Cx43+/- hearts had at least 1 run of sustained VT that lasted >3 seconds. The majority of these runs persisted for >20 seconds. In contrast, only 4 Cx43+/+ hearts had at least 1 run of sustained VT, and only 1 of these runs lasted >20 seconds.

Significant differences in the incidence, frequency, and duration of spontaneous and inducible VT occurred in the 2 groups (Figures 2B and C). Spontaneous VT occurred in more than twice as many Cx43+/+ as +/- hearts (Figure 2B). Nine of 16 Cx43+/- hearts had multiple runs of spontaneous VT compared with only 1 of 16 Cx43+/+ hearts (P<0.01); 8 Cx43+/+ hearts had sustained runs of VT compared with only 1 Cx43+/- heart (P<0.01). Pacing-induced ventricular tachycardias were observed in more Cx43+/+ than +/- hearts (P<0.01), and a greater number of Cx43+/+ hearts exhibited multiple runs of inducible VT (P<0.05) (Figure 2C).

The time of onset of the first spontaneous VT after LAD occlusion occurred significantly earlier in Cx43+/- hearts (P<0.05) (Figure 3). Of the 9 Cx43+/- hearts that developed spontaneous tachycardia, 7 exhibited the first run of VT during the first 12 minutes after coronary artery occlusion. In contrast, the earliest onset of spontaneous VT in any Cx43+/+ heart did not occur until after 13 minutes of ischemia.

**PVBs in Cx43+/- and Cx43+/+ Hearts**

At least 1 PVB occurred at some point during the 60-minute interval of coronary occlusion in nearly all (15 of 16 Cx43+/- and 14 of 16 +/-) ischemic hearts, but the number of PVBs differed markedly between the 2 groups. The number of PVBs was counted for each preparation during each of the four 15-minute intervals of the entire 60-minute period of coronary occlusion. A maximum of 20 PVBs was tabulated for each 15-minute interval, although the presence of a greater number was noted. Generally, when the threshold of 20 PVBs was achieved, more than 20 PVBs occurred. As shown in Table 2, 2 to 4 times as many Cx43+/- as Cx43+/+ hearts exceeded the threshold of ≥20 PVBs during each 15-minute interval, but because sample sizes were small, only the difference at the 45- to 60-minute interval achieved statistical significance. However, 11 of 16 Cx43+/- compared with only 5 of 16 +/- hearts developed ≥20 PVBs during any 15-minute interval (P<0.05). Furthermore, 6 of 16 Cx43+/- hearts exceeded the threshold in 3 or all 15-minute intervals compared with only 1 of 16 Cx43+/+ hearts (P<0.05).

**Discussion**

Altered electrical coupling has been strongly implicated in the development of arrhythmias induced by ischemia,1–4,9–12 but because multiple pathological responses to ischemia occur concomitantly, it has not been previously possible to elucidate the specific contributions of changes in coupling to arrhythmogenesis in acute myocardial ischemia. Comparison of wild-type and Cx43-deficient hearts allowed us to directly assess the effect of diminished Cx43 expression on arrhythmogenesis induced by ischemia without altering other influential factors at the same time. Our results provide strong, direct evidence that diminished electrical coupling caused by

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<th>TABLE 2. No. of Hearts Exhibiting ≥20 PVBs During 15-Minute Intervals of Ischemia</th>
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<tr>
<td>Minutes After Coronary Occlusion</td>
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<tr>
<td>0–15 15–30 30–45 45–60 15-min Interval 15-min Intervals</td>
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<tr>
<td>Cx43+/- (n=16)</td>
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<td>Cx43+/+ (n=16)</td>
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P 0.10 0.12 0.12 0.002 0.03 0.03
reduced expression of Cx43 accelerates the onset and increases the incidence, frequency, and duration of ventricular arrhythmias after acute regional ischemia. Although we have not formally excluded the possibilities that Cx43+/− hearts have a defect in another ion channel that becomes manifest only during ischemia or that adult Cx43+/− and +/+ myocytes may differ in active membrane properties, comparisons of action potential parameters and quantitative measures of voltage-gated Na+ channel protein expression and activity have revealed no differences in ventricular myocytes from neonatal Cx43+/− and +/+ mice.5,13

Ischemia-Induced VT and Altered Intercellular Coupling
Defining the role of specific gene products in arrhythmogenesis in mouse models of human diseases will require elucidation of arrhythmia mechanisms. Although analysis of arrhythmia mechanisms in mice will be technically challenging, Vaidya et al.14 have observed stationary vortexlike reentry on the surface of a mouse heart induced by burst pacing and manifest in the ECG as sustained monomorphic tachycardia. Our observations in the present study are consistent with the hypothesis that reentrant circuits in Cx43+/− hearts may be more stable and inductive than in Cx43+/+ hearts. Modest conduction slowing under basal conditions in Cx43+/− hearts probably reduced the wavelength for reentry and further enhanced development of stable reentrant circuits when progressive regional uncoupling was induced by coronary occlusion. In a few cases, prolonged spontaneous tachycardias were easily terminated by a single extrastimulus, suggesting that the arrhythmias may have been reentrant. It should be stressed, however, that at least some of the arrhythmias observed in the present study could have arisen by nonreentrant mechanisms such as triggered activity, a mechanism that has been implicated in some monomorphic ventricular tachyarrhythmias in patients without organic heart disease.15,16

Accelerated onset of spontaneous VT in Cx43+/− mice supports the concept of a critical threshold of uncoupling in arrhythmogenesis. Cx43+/− hearts probably achieved a pathophysiological level of uncoupling and conduction slowing more rapidly than did Cx43+/+ hearts. Arrhythmias induced by acute ischemia occur in 2 phases, an initial wave (Ia) related in part to altered extracellular K+ levels, and a later wave (Ib) occurring 12 to 30 minutes after the onset of ischemia and related to electrical uncoupling.9–11,17 The time course of electrical uncoupling in response to ischemia has not been reported in mice, but the time course of arrhythmias observed in Cx43+/+ hearts is reminiscent of type Ib arrhythmias in pig hearts.11 Future studies will be required to determine whether the time course of uncoupling induced by ischemia differs in Cx43+/− and +/+ hearts.

Ischemia-Induced PVBs and Intercellular Coupling
Significantly more Cx43+/− than +/+ hearts had ≥20 PVBs in at least one of the four 15-minute intervals and during 3 or all 15-minute intervals. Hearts that exceeded the threshold of 20 PVBs generally exhibited many more premature beats. Thus differences between Cx43+/− and +/+ hearts in the frequency of PVBs would undoubtedly have been greater if the actual number of PVBs had been counted. There was also a clear trend toward earlier onset of PVBs in ischemic Cx43+/− hearts. Premature ventricular beats may arise by reentry or be initiated by triggered activity. In the latter mechanism, a focal depolarization must be propagated to produce the premature beat. We do not yet know the mechanism responsible for the apparently greater number and earlier onset of PVBs in Cx43+/− mice after coronary occlusion, but our observations provide direct experimental evidence implicating altered coupling in the development and time of onset of PVBs during ischemia. The observations also raise intriguing questions about the potential pathophysiological links between changes in coupling and the development and propagation of PVBs initiated by either reentry or triggered activity.

Recent experimental observations and computer modeling studies have begun to delineate the effects of electrical coupling on both the initiation and propagation of triggered events.18–22 For example, Saiz et al.22 showed in a modeling study that a moderate amount of uncoupling between normal myocardium and a region conducive to formation of early afterdepolarizations (EADs) (such as an area of acute ischemia) directly enhances both initiation of EADs and spread to neighboring tissue. They found that a specific degree of uncoupling could prolong repolarization and promote the generation of EADs and allow for a critical rise in membrane potential to achieve transfer of the impulse to the surrounding tissue. Our finding of increased PVBs in Cx43-deficient hearts is consistent with these computer models and provides
experimental evidence potentially linking alterations in coupling with the generation and propagation of EADs. Future studies will be required to confirm the relation between diminished coupling and the development of PVBs to elucidate the specific mechanisms (reentry and/or triggered activity) responsible for PVBs and to characterize potential pathophysiological links between the development of PVBs and VT in mice with different baseline levels of intercellular coupling.

**Clinical Implications**

In view of the findings of the present study, it is logical to suggest that interventions designed to prevent uncoupling could diminish arrhythmogenesis during ischemia. Although such a strategy could limit the development of slow conduction and retard formation of arrhythmia substrates, it also could counteract potential benefits of uncoupling healthy and ischemic myocytes from each other. In this setting, uncoupling may be thought of as an adaptive process that isolates irreversibly injured cells from viable neighbors. Acute uncoupling may also electrically silence viable but injured regions and limit their contribution to arrhythmias dependent on slow conduction and unidirectional conduction block. Further investigation will be required to elucidate the potential benefits and risks of modulating cell-to-cell coupling and define the effects of total versus partial uncoupling in the pathogenesis of ischemia-induced arrhythmias.

**Acknowledgment**

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