Atrial tachycardia/fibrillation in the connexin 43 G60S mutant (Oculodentodigital dysplasia) mouse

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Tuomi JM, Tyml K, Jones DL. Atrial tachycardia/fibrillation in the connexin 43 G60S mutant (Oculodentodigital dysplasia) mouse. Am J Physiol Heart Circ Physiol 300: H1402–H1411, 2011. First published January 14, 2011; doi:10.1152/ajpheart.01094.2010.—Atrial fibrillation (AF), the most common cardiac arrhythmia seen in general practice, can be promoted by conduction slowing. Cardiac impulse conduction depends on gap junction channels, which are composed of connexins (Cxs). While atrial Cx40 and Cx43 are equally expressed, AF studies have primarily focused on Cx40 reductions. The G60S Cx43 mutant (Cx43G60S+/−) mouse model of Oculodentodigital dysplasia has a 60% reduction in Cx43 in the atria. Cx43G60S+/− mice were compared with Cx40-deficient (Cx40−/−) mice to determine the role of Cxs in atrial tachycardia/fibrillation (AT/F). Intracardiac electrophysiological studies were done in 6-month-old male C57BL/6 Cx43G60S+/− mutant, littermate (Cx43+/−), Cx40−/−, and C57BL/6 wild-type (WT) mice. AT/F induction used an extra stimulus during sinus rhythm, programmed electrical stimulation, or burst pacing (1-ms pulses, 50-Hz, 400-ms train) in the presence and absence of carbachol (CCh). Atrial effective refractory periods did not differ between strains. Cx43G60S+/− mice were more susceptible to induction of sustained AT/F (duration >2 min, 9 of 12; maximum >35 min) compared with Cx43+/− mice (3 of 11; χ2 = 5.24; P = 0.02). CCh enhanced sustained AT/F susceptibility in WT (from 1 of 12 without, to 7 of 10 with CCh; χ2 = 8.98; P < 0.01) but not in Cx40−/− mice (1 of 13 without vs. 2 of 9 with CCh; χ2 = 0.95; P = NS). The pattern of epicardial recordings during AT/F in Cx43G60S+/− mice was left preceding right, with left atrial fractionated activation patterns consistent with clinical observations of AF. In conclusions, while Cx43G60S+/− mice had severe AT/F, Cx40−/− mice were resistant to CCh-induced AT/F.

gap junctions; murine; arrhythmia; connexin 40; cholinergic

Atrial fibrillation (AF) is the most common cardiac arrhythmia seen by the general practitioner (3). It is characterized by disorganized and rapid atrial electrical activation, which can drive rapid and irregular ventricular activation. AF requires a trigger for initiation, together with a dynamic susceptible or vulnerable substrate for maintenance. It has been shown to be triggered by either wavebreak (24) or ectopic foci (22) arising from regions such as the pulmonary veins (20) or the posterior left or right atrium (20, 36). Triggers combined with dynamic substrates of structural heterogeneities, reduced refractoriness, enhanced spatial dispersion of refractoriness, and abnormal impulse conduction to initiate and perpetuate the arrhythmia (38).

Gap junction channels are critical for the conduction of electrical impulses in the heart and may be involved in arrhythmia. They are composed of four transmembrane domain proteins, connexins (Cxs), which oligomerize in the Golgi apparatus to form hexamers called connexons (31). Connexons from adjacent cells dock, primarily at intercalated disc, to form low resistance channels that allow rapid propagation of electrical signals (7). While four primary isoforms of Cxs are found in the heart (mouse Cx30.2/human Cx31.9, Cx40, Cx43, and Cx45), only Cx40 and Cx43 are abundant in the working myocardium. The atrium expresses similar amounts of Cx40 and Cx43 (33).

Remodeling of connexins can be functional (altered conduc-
tance) and structural (altered content/distribution) (14). The role of connexins in AF has been studied in animal models and patients with AF; however, the exact role of Cx40 and Cx43 is not clear (14). In wild-type (WT) mice following the administration of carbachol (CCh), seven of eight mice were susceptible to atrial tachycardia/fibrillation (AT/F) lasting 139.2 ± 402.1 s (51). However, studies in Cx40−/− mice have reported that AT/F was not detected (1), occurred infrequently (5 of 19; Ref. 4), had no difference in susceptibility compared with WT mice (42), or was short lived (5/10, <1 s; Refs. 4, 19, 49). These reports demonstrate that relative to WT mice in the presence of CCh (51), Cx40−/− mice have little susceptibility to sustained AT/F. However, somatic mutations in Cx40 (17) and Cx43 (44) have been reported in patients with idiopathic AF. When expressed in N2A cell pairs, similarly mutated Cx40 had reduced junctional conductance and inhibited WT Cx43 junctional conductance. These observations highlight an important distinction between functional and structural alterations of gap junctions.

Arrhythmia studies in transgenic mice have primarily focused on Cx40-deficient (Cx40−/−) mice as Cx43-deficient (Cx43−/−) mice die perinatally (40). The cardiac selective Cx43 knockout mouse has only been used in studies of ventricular arrhythmia (18). To date, 62 mutations in Cx43 have been linked to Oculodentodigital dysplasia (ODDD), a pleitropic, autosomal dominant disorder in humans (39, 44) primarily affecting the eye, dentition, and digits of the hands and feet. Cardiac defects were reported in 177 individuals from 54 families with ODDD (39). Both G138R (13) and I130T (27) Cx43 mutant mice with the ODDD phenotype have increased susceptibility to ventricular arrhythmia; however, atrial arrhythmia susceptibility was not determined. The Cx43 G60S (Cx43G60S+/−) mutation in mice (39) is dominant negative, causing connexons with mutant and WT Cx43 to be retained in the Golgi and targeted for degradation (35). Cx43G60S+/− mutant mice have a 60% reduction in total atrial Cx43 protein.

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content, with a preferential downregulation (~80%) of the highly phosphorylated (functional) form of Cx43 (35). The Cx43G60S/+ mutation did not alter Cx40 or Cx45 expression. We took advantage of this well-characterized Cx43G60S/+ mouse model to determine the role of Cx43 reductions in AT/F. Intracardiac electrophysiological pacing determined AT/F susceptibility in 6-mo-old male mice. As no atrial arrhythmias were reported in WT mice and a relatively low frequency of AT/F in Cx40-/- mice, we compared Cx43G60S/+ mutant to age-matched Cx40-/- mice to determine the role of reduced Cx43 and total loss of Cx40 in AT/F.

METHODS

Animals

Animal studies were approved by the Animal Use Committee of the University of Western Ontario (protocol #2006-121-12) and complied with the Guidelines of the Canadian Council on Animal Care and the Guide to the Care and Use of Laboratory Animals published by the National Institutes of Health (NIH Publication No. 85-23, revised 1996). Six-month-old male Cx43G60S/+ mice were obtained from the colony maintained by Dr. Gerald Kidder that was originally developed by Dr. Janet Rossant (Centre for Modeling Human Disease, Toronto, ON, Canada; Ref. 16). Comparisons were made to age-matched male C57BL/6 littermate (Cx43+/+), WT, and Cx40-/- mice originally derived from male mice provided by Dr. David Paul (Harvard University, Boston, MA; Ref. 43).

Mice were anesthetized with an intraperitoneal injection mixture of ketamine (150 mg/kg) and xylazine (10 mg/kg). Body temperature was monitored with a VSI-402 (Yellow Springs Instruments, Yellow Springs, Ohio) rectal probe inserted 1–1.5 cm beyond the anal sphincter and maintained at 36.5–38°C (45). A 23-gauge Teflon endotracheal tube was inserted and ligated in place to keep the airway open.

Surface ECGs were obtained using four subcutaneous, 25-gauge platinum electrodes (Grass Instrument, Quincy, MA) placed at the base of each limb. ECG tracings were filtered between 0.05 to 100 Hz and digitized and sampled at 1.5 kHz with an ECG100 preamplifier connected to a MP100 recording system (BIOPAC Systems, Biolyx, Montréal, PQ, Canada). Blood pressure and simultaneous heart rate were assessed noninvasively in conscious WT and Cx40-/- mice using the CODA system (15).

A 2-F octapolar stimulation/recording/drug infusion catheter (CIB’ER Mouse, NuMED, Hopkinton, NY) was inserted through the right jugular vein and advanced into the right atrium and ventricle. Intracardiac electrograms were filtered between 100 to 5,000 Hz (DA100 amplifier; BIOPAC Systems) and sampled at 1.5 kHz. All data were recorded using a personal computer running Acknowledge (DA100 amplifier; BIOPAC Systems) and sampled at 1.5 kHz. All data were expressed as the means ± SE.

RESULTS

Standard Electrophysiological Parameters

Cx40-/- mouse ECGs had prolonged P-wave and QRS durations, and QT and PQ intervals compared with age-matched WT mice (Table 1). Atrial-Hisian (AH) intervals were shorter during sinus rhythm and at a drive cycle length of 100 ms (AH100), while His-ventricular intervals were prolonged compared with WT mice (Table 1). Wenkebach cycle lengths were also shorter in Cx40-/- mice.

Cx43G60S/+ mice had no differences in P-wave durations or PQ intervals compared with age-matched littermates (Cx43+/-); however, QRS durations and QT intervals were prolonged (Table 2). AH intervals recorded during sinus rhythm did not differ significantly, but with pacing, AH100 was lower in the Cx43G60S/+ mice compared with Cx43+/- mice. His-ventricular intervals were also shorter in the Cx43G60S/+ mice (Table 2).

Effective refractory periods. PES consistently revealed regional heterogeneity in AERPs: longer in the high right than the mid-right atrium (Tables 1 and 2) but did not differ between strains. CCh reduced sinus rate (~20%) and AERPs in Cx40-/- and WT mice (Table 1). Interestingly, the VERP100 was lower in Cx43G60S/+ compared with Cx43+/- mice, consistent with results from isolated right ventricular myocytes of the conditional Cx43 knockout mouse, which was found to have enhanced IKr activity (10). For Cx40-/- mice, VERP did not differ from those of WT mice; however, following CCh administration, VERP100 in WT were even higher than those of Cx40-/- mice (Table 1).

Arrhythmia Induction

Three pacing protocols were used to evaluate the susceptibility to electrically induced AT/F: 1) a single atrial stimuli (SAS) delivered during sinus rhythm, timed to follow the P wave (Fig. 1A); 2) PES, with an increasingly premature extra stimuli (S2; Fig. 1B) (26); and 3) atrial burst pacing (1-ms pulses, 50-Hz, 400-ms train) (25, 45). AT/F was characterized based on the susceptibility to the pacing modality and the arrhythmia duration: a) no response; b) lasting <10 s; c) lasting from 10 to 60 s; and d) >120 s.

We previously determined that AT/F, when inducible in WT mice, is dependent on the pacing location in the right atrium, validated by using the His bundle potential as a landmark of catheter positioning (45). Previous murine studies (51) of atrial arrhythmia frequently report low susceptibility to AT/F in WT mice in the absence of CCh. As electrically induced arrhythmia was rare in Cx40-/- mice, additional provocation used the nonselective cholinergic agonist CCh (0.5 mg/kg ip; Sigma, Mississauga, ON) in WT and Cx40-/- mice (45).

A subgroup of Cx43G60S/+ mice was mechanically ventilated at a respiratory rate of 100 breaths per minute. The chest was open via a lateral thoracotomy between the first and second ribs. For epicardial recordings, Teflon-insulated silver bipolar electrodes (0.2 mm spacing) were positioned on the surface of the left and right atrial appendages or the posterior left atrium (23).

Statistical Analysis

Drug and strain comparisons were analyzed with two-way ANOVA and the Bonferroni post hoc test (Prism 4.0; GraphPad Software, La Jolla, CA). The chi square test was used to analyze discrete data. A probability of P < 0.05 was considered statistically significant. All data are expressed as the means ± SE.
Cardiac Hypertrophy/Hypertension

Cardiac hypertrophy (29) and hypertension (50) were reported in Cx40\textsuperscript{H11002}/H11002/H11002 mice. As both hypertension and heart failure are risk factors for developing AF, we documented these phenotypes in our mice. Cx40\textsuperscript{H11002}/H11002/H11002 mouse hearts were visibly hypertrophic with a 54% increase in the ventricular weight-to-tibial length ratio (10.8 ± 0.8 vs. 7.0 ± 0.4 mg/mm; \( P < 0.05 \)), while Cx43\textsuperscript{G60S}\textsuperscript{H11001} mouse hearts were normal (6.9 ± 0.5 vs. 7.2 ± 0.7 mg/mm; \( P = \text{NS} \)). With the use of tail cuff measurement, conscious Cx40\textsuperscript{H11002}/H11002/H11002 mice had higher systolic (179.3 ± 4.8 vs. 123.1 ± 5.6 mmHg; \( P < 0.001 \)) and diastolic (140 ± 7.3 vs. 94.3 ± 5.8 mmHg; \( P < 0.001 \)) blood pressures and lower heart rates (594.1 ± 28 vs. 701.2 ± 36 beats/min; \( P < 0.05 \)) compared with age-matched WT mice. One Cx40\textsuperscript{H11002}/H11002/H11002 animal died suddenly from unknown causes during tail cuff measurement. This death may have resulted from restraint stress-induced ventricular arrhythmia (37), as no WT but two Cx40\textsuperscript{H11002}/H11002/H11002 mice had burst pacing induced ventricular tachycardia lasting ~13 s. Of note, atrial standstill was reported in patients that co-inherit a novel SCN5A mutation along with a Cx40 polymorphism (34). Blood pressures in Cx43\textsuperscript{G60S}\textsuperscript{H11001} mice were not determined.

Atrial Tachyarrhythmia Induction

Pacing-induced atrial arrhythmia had electrophysiological characteristics of AF (25), although distinguishing from rapid atrial tachycardia and validation of AF would require high density mapping. Thus, we labeled all atrial arrhythmias as AT/F (45).

C57BL/6 WT mice were relatively insensitive to any duration of AT/F induction (0.1 s to >120 s) with PES (4 of 12, 33%), while they were inducible with burst pacing (9 of 12, 75%; Fig. 2A). CCh injection (50 ng/g ip) caused a trend to increase susceptibility to AT/F induction with both PES (7 of 10, 70%; \( \chi^2 = 2.93; P = 0.08 \)) and burst pacing (9 of 10, 90%; \( \chi^2 = 0.83; P = 0.36 \)). SAS induction protocol was not performed in WT mice. However, CCh injection prolonged the duration of AT/F with only 1 of 12 untreated WT mice having sustained AT/F (>2 min) before, while 7 of 10 had sus-
Table 1. Intracardiac electrophysiological values recorded in the absence and presence of carbachol in 6-mo-old male Cx40$^{-/-}$ and C57BL/6 wild-type mice

<table>
<thead>
<tr>
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<tr>
<td></td>
<td>Value, ms</td>
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<td>Value, ms</td>
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<tr>
<td>P wave</td>
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<tr>
<td>QRS</td>
<td>15 ± 1</td>
<td>13</td>
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<tr>
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<td>47 ± 1</td>
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<td>29 ± 2</td>
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<tr>
<td>AMERP$_{100}$</td>
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<td>13</td>
<td>26 ± 1</td>
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<td>13</td>
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<tr>
<td>AH</td>
<td>28 ± 1</td>
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<td>23 ± 1</td>
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<tr>
<td>HV</td>
<td>11 ± 1</td>
<td>13</td>
<td>20 ± 1</td>
</tr>
<tr>
<td>VERP$_{100}$</td>
<td>46 ± 2</td>
<td>12</td>
<td>42 ± 2</td>
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Table 2. Intracardiac electrophysiological values recorded from 6-mo-old male Cx43$^{G60S/+}$ and Cx43$^{+/-}$ mice

<table>
<thead>
<tr>
<th></th>
<th>Cx43$^{G60S/+}$</th>
<th>Cx43$^{+/-}$</th>
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<tr>
<td></td>
<td>Value, ms</td>
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<tr>
<td>P wave</td>
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<td>AHERP$_{100}$</td>
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<tr>
<td>AMERP$_{100}$</td>
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<td>10</td>
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<tr>
<td>AVNERP$_{100}$</td>
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<td>10</td>
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<tr>
<td>AH$_{100}$</td>
<td>37 ± 1</td>
<td>11</td>
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<tr>
<td>AH</td>
<td>27 ± 1</td>
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<tr>
<td>HV</td>
<td>10 ± 1</td>
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<tr>
<td>VERP$_{100}$</td>
<td>40 ± 1</td>
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Values are means ± SE; n, number of mice. *P < 0.05 for Cx43$^{G60S/+}$ vs. Cx43$^{+/-}$ mice.
Thus we determined the efficacy of intravenous darifenacin hydrobromide (1 mg/kg), a selective M3 muscarinic receptor antagonist, to terminate the "new onset" sustained AT/F in 5 Cx43G60S/H11001 mice. Intravenous injection of darifenacin terminated sustained AT/F in all five mice. Of note, AT/F was reinducible with burst pacing 5 min after drug treatment.

Electrogram Analysis

The morphology of bipolar electrograms has been extensively studied in patients and animal models of AF (11, 30). Normal unfractionated bipolar electrograms usually have a single monophasic or biphasic potential, the morphology of which depends on the orientation of the electrode poles relative to the vector of the propagating electrical wave. Fractionated electrograms have multiple deflections within one cardiac cycle. As the study of electrogram fractionation in AT/F, to our knowledge, has not previously been performed in the mouse, we present the spectrum of electrograms that we have observed (Fig. 4). The simplest fractionated electrograms was a double potential, in which the time between peaks could be short (short double), or long (long double) (Fig. 4). Complex fractionated atrial electrograms have multiple peaks or may have continuous electrical activity throughout the entire cardiac cycle (Fig. 4). In humans, fractionated electrograms may be found in the following: 1) in areas with slow conduction, such as the border zone of an infarct; 2) associated with a line of block (short/long double) (11); 3) around the pivot point of a micro reentrant circuit; or 4) where waves collide (30). Complex fractionated atrial electrograms have also been observed in myocyte monolayers, at sites of migrating rotors and wave break (46). As an index of conduction disturbance in vivo, bipolar right atrial activation patterns observed in the MRA and HRA during AT/F were used, since optical mapping is not available in our laboratory, and in mice, optical mapping usually requires ex vivo studies. We devised an index of electrogram patterns during AT/F, based on the degree of conduction disturbance: a) the lowest level was designated “fractionation only,” without a variable HRA cycle length compared with that recorded from the MRA; b) the intermediate had a variable HRA cycle length compared with the MRA cycle length, with or without intermittent activation failure in the HRA; and c) the highest level had a pattern of 2:1 activation failure in the HRA compared with that recorded in the MRA.

WT and Cx43G60S/H11001 mice commonly had unfractionated single potentials recorded from both the MRA and HRA electrodes during both sinus rhythm and AT/F. Fractionated double potentials and complex electrograms were observed during AT/F in Cx43G60S/H11001 mice, commonly with double potential morphologies, which may have been due to functional conduction block along a zone of poor conduction.

To determine if the Cx43 G60S mutation-induced reduction in Cx43 was associated with disturbed conduction during AT/F (Fig. 4). The simplest fractionated electrograms was a double potential, in which the time between peaks could be short (short double), or long (long double) (Fig. 4). Complex fractionated atrial electrograms have multiple peaks or may have continuous electrical activity throughout the entire cardiac cycle (Fig. 4).
arrhythmia driver was in the left atrium (Fig. 6A). Spontaneous termination, when observed, occurred in the left with the last area of activation in the right atrium. Recordings from the posterior left atrium also revealed zones of high frequency complex fractionation (Fig. 6B), consistent with AF.

**DISCUSSION**

This main purpose of this study was to investigate the role of the Cx43 G60S mutation on the susceptibility to atrial tachyarrhythmias in the mouse. Studies in the Cx40−/− mouse also allowed direct comparisons on the role of reductions in each Cx isoform in AT/F. The results indicate that Cx43G60S/+ mice have increased susceptibility to AT/F while Cx40−/− mice do not.

As gap junctions are critically important for cardiac impulse propagation, it is not surprising that alterations of Cxs would be important in atrial arrhythmia. However, as Cx43-deficient mice die prematurely, comparative in vivo studies on the role of Cx40 and Cx43 in AT/F have not been performed. Interpretations of the significance of the results of past studies in Cx40−/− mice have been hindered by the lack of definition of what constitutes a sustained atrial tachyarrhythmia in the mouse. In the presence of CCh, WT mice had AT/F, averaging >139 s (51). In the present study, we found long-lasting (>2 min, and as long as 35 min), readily inducible (100% susceptibility) AT/F in Cx43G60S/+ mice. These observations provide a benchmark to define “sustained AT/F” in the mouse, allowing differentiation from transient arrhythmias. We observed infrequent, primarily short runs of rapid atrial activity following burst pacing in the Cx40−/− mice with only one incident of sustained AT/F, consistent with previous findings. After we observed AT/F lasting >35 min in the Cx43G60S/+ mutant mice, it has become clear that short duration (<1 s) events are likely of limited physiological significance. To evaluate if Cx40 deficiency could make the atrium more susceptible to AT/F in the presence of another provocative agent, we determined the susceptibility to CCh-induced AT/F in the Cx40−/− mouse. However, we found that CCh did not significantly enhance inducibility of sustained AT/F in Cx40−/− mice (>120 s; Fig. 3C). This finding is consistent with those of dogs treated with n3-polysaturated fatty acids, which had a reduced incidence of vagally induced AF, where protection was related to n3-polysaturated fatty acid induced reductions in Cx40 protein expression (41).

Three different pacing protocols were used to induce AT/F: burst pacing, PES with a single premature extra stimulus, and SAS with single atrial stimuli delivered during sinus rhythm timed to follow the P wave and interact with the heterogeneous

**Left Atrial Recordings**

As AF in patients frequently originates from the left atrium/pulmonary vein region, we performed epicardial left and right atrial appendage recording in a subset of mechanically ventilated Cx43G60S/+ mice (n = 4). In all mice, atrial activation followed a left leading right atrial pattern, indicating that the
refractoriness of the atrial tissue. Both PES and SAS were thought to mimic physiological initiation of AT/F, as premature atrial beats often precede AT/F episodes (22). Burst pacing, perhaps a nonphysiological provocation, was found to more reliably induce AT/F by promoting cardiac electrical instability (25). Susceptibility to AT/F in the mouse induced by single stimuli delivered in sinus rhythm, an extremely mild pacing protocol, has not previously been reported in the mouse. AT/F induction with the SAS protocol indicates that the Cx43G60S/+H11001 mice are extremely vulnerable to atrial arrhythmia. Detailed electrogram analysis showed that underlying heterogeneities in AERPs (in MRA vs. HRA) on a background of reduced Cx43 content were associated with fibrillatory conduction with short cycle lengths (Fig. 5). Mechanistically, AF may result from a single source (mother rotor) resulting in fibrillation due to the breakup of high frequency wavefronts interacting with tissue heterogeneities (24). Thus reductions in Cx43 could promote AF initiation by promoting a wavebreak, coupled with conduction disturbances (48) to perpetuate AF. In an effort to compare murine AT/F to human AT/F, we determined if the mouse had left atrial predominance in AT/F mechanisms as had been observed in humans. Epicardial left atrial recordings in mechanically ventilated Cx43G60S/+ mice during AT/F had a left preceding right atrial activation pattern and displayed zones of complex fractionation in the posterior left atrium. This result is consistent with clinical findings where the driver of AF is commonly observed in the posterior left atrium/pulmonary vein region (20, 36). While sustained AT/F occurred infrequently in the Cx40−/− mice, genetic polymorphisms in Cx40 are associated with AF (6, 8). While a functional change in gap junctions caused by retaining mutant Cx40 at the intercalated disc may promote AF (17), the present data show that complete removal of Cx40 (structural change) appears to provide resistance to CCh-induced AT/F. However, a large reduction (~80%) of phospho-Cx43 (the functional isoform of Cx43 present at the intercalated disc) promotes...
enhanced AT/F susceptibility. While these results warrant further study, they are consistent with previously published results.

The atrium has equivalent expressions of Cx40 and Cx43. Thus atrial gap junctions may have varying stoichiometry (homomeric, heteromeric, homotypic, or heterotypic), with unique gating and conductance properties (9). In A7r5/Rin cell pairs, Cx40 and Cx43 can form homomeric/heterotypic and heteromeric/heterotypic gap junctions with unique gating and conductance properties (9); however, junctional conductance is reduced relative to homomeric/homotypic channels. Hela cell pairs coexpressing Cx40 and Cx43 were also reported to have substantial reductions (~56–66%) in junctional conductance compared with homotypic cell pairs (47). In addition, Haubrich et al. (21) found that homomeric Cx40 and Cx43 hemichannels are incompatible, as conduction was not detected (21). It is reasonable to assume that Cx40−/− and Cx43G60S/+ mice would have altered stoichiometry of connexon oligomerization, which would alter conduction properties. However, the presence, and functional consequence, of gap junctions of mixed stoichiometry in the atrium has been reported to be uncertain (12).

A functional consequence of coexpression of Cx40 and Cx43 on junctional conductance in the mouse atrium has been studied using spermine, a compound that is reported to dose dependently and selectively block Cx40 containing gap junctions (33). Spermine blockade in Cx40−/− and Cx40+/+ atrial cell pairs indicated that Cx40 contributes to ~40% of WT junctional conductance (33). Susceptibility to spermine inhibition also suggested that ~10–20% of atrial junctional conductance might be due to gap junctions of mixed stoichiometry (33). Also, junctional conductance was unaltered in myocyte pairs from Cx40−/− mice (WT = 6.03 ± 1.09 nS vs. Cx40−/− = 6.10 ± 1.14 nS) [see Supplementary Material in Lin et al. (33)], suggesting an important role for channels of mixed stoichiometry or that Cx43 may compensate for the lack of Cx40.

Cx43G60S/+ mice have a ~80% reduction in ventricular phosphorylated Cx43 but only a ~50% decrease in ventricular myocyte junctional conductance. This suggests that myocytes biosynthesize larger amounts of Cx43 than is necessary to maintain normal cardiac junctional conductance function (35). This idea is consistent with the observation that in ventricular myocytes only 10% of the available cardiac junctional conductance channels are open to conduct current during a given action potential (52). Both phosphorylation and pH are well known to regulate functional properties of gap junction channels. Ischemia results in acidification and closure of homomeric Cx40 and Cx43 containing gap junctions. However, coexpression of Cx40 and Cx43 in Xenopus oocytes enhances the pH sensitivity of the channel, a phenomenon that requires the carboxy termini of both connexins (5). Reductions in either Cx40 or Cx43, by reducing heterogeneity, would then likely also decrease the pH sensitivity of the remaining gap junction channels.

In synthetic strands of neonatal atrial myocytes from Cx40+/− and Cx43−/− mice, conduction velocity was increased in Cx40+/− strands but was reduced in Cx43−/− strands (2). The percent cell area occupied by the Cx40 immunosignal was smaller (2.0 ± 1.6 vs. 1.0 ± 0.2%) in Cx43−/− cultures, while the area of Cx43 immunosignal was larger (1.2 ± 0.9 vs. 3.1 ± 3.6%) in Cx40−/− cultures (2). This suggests that Cx40 may sterically limit the number of Cx43 containing gap junctions at the intercalated disc in WT atria. Removal of Cx40 would allow an increased insertion of Cx43 containing gap junctions into the intercalated disc region to compensate for the reduced Cx40. Compensation may not be limited to those of neonatal myocytes, as optical mapping from adult Cx40−/− mice revealed that morphologically normal hearts have no change in atrial conduction velocity (32). Similar observations have been made in human myocardium, where there is a positive correlation between conduction velocity and the relative quantity of Cx immunolabeling [expressed as Cx40/(Cx40 + Cx43)]; when the Cx40 signal was higher the Cx43 signal was lower, conduction velocity was lower (28).

Taken together, these observations suggest the following theoretical model of the role of alterations of connexins in the atrium. As stated above, Cx40 may sterically limit Cx43 content at the intercalated disc region; thus loss of Cx40 would lead to compensatory increased Cx43 insertion; however, the reverse seems not to occur. Atrial gap junction channel conductance follows a hierarchy where Cx40/Cx40 > Cx43/Cx43 > Cx40/Cx43. Therefore, homogenous loss of Cx40 leads to increased junctional conductance (plus decreasing pH sensitivity of channels) and reduced heterogeneity as only Cx43/Cx43 gap junctions would remain. As such, decreased heterogeneity of coupling might then reduce AT/F. On the other hand, somatic mosaic mutation in either Cx40 or Cx43 in patients leads to AF. This is likely due to the resultant enhanced heterogeneity of the atrial tissue accentuated by the mosaic nature of the functional changes, whether or not there is an accompanying localization in the intercalated disc.

In conclusion, it is clear that alterations in gap junctions can have a role in AF mechanisms. However, that role may not simply be due to reductions in Cx content (14). Proper interpretation of the role of Cxs in AT/F should consider the functional alterations, structural alterations, stoichiometric alterations, and the potential for compensation (14). In this study, we found that the Cx43G60S/+ mutant mouse had sustained AT/F. We also noted that Cx40+/− mice were resistant to CCh-induced AT/F despite severe hypertension and cardiac hypertrophy; both risk factors for development of AF. As AF can be a silent disease, observed only after diagnostic (ECG) testing, it may be wise to monitor patients with Cx43 mutations causing ODDD, as there are reports of cardiac phenotypes (39) in these patients and they may have an additional age-related risk for developing AF.

Limitations

It may be difficult to distinguish AF from atrial tachycardia, when evaluating recordings from the mouse heart, particularly with arrhythmia of short duration (45). However, with the use of the standard clinical criteria for identification of AF, lack of regular P waves on ECGs and irregularly irregular ventricular responses, often with left atrial predominance of AF mechanisms, in this study it was often possible to demonstrate these classical characteristics of AF in the mouse. In addition, it may not always be possible to extrapolate data obtained from the mouse to explain arrhythmia mechanisms in the human.
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DISCLOSURE

No conflicts of interest, financial or otherwise, are declared by the author(s).

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