Published in final edited form as:

Am J Physiol Heart Circ Physiol. 2007 July ; 293(1): H182–H192. doi:10.1152/ajpheart.00684.2006.

Cardiac-restricted angiotensin-converting enzyme overexpression causes conduction defects and connexin dysregulation

Vijaykumar S. Kasi^{1,*}, Hong D. Xiao^{3,*}, Lijuan L. Shang¹, Shahriar Iravanian¹, Jonathan Langberg², Emily A. Witham¹, Zhe Jiao¹, Carlos J. Gallego¹, Kenneth E. Bernstein³, and Samuel C. Dudley Jr.¹

¹Division of Cardiology, Atlanta Veterans Affairs Medical Center and Emory University, Atlanta, Georgia

²Division of Cardiology, Emory University, Atlanta, Georgia

³Department of Pathology and Laboratory Medicine, Emory University, Atlanta, Georgia

Abstract

Renin-angiotensin (RAS) system activation is associated with an increased risk of sudden death. Previously, we used cardiac-restricted angiotensin-converting enzyme (ACE) overexpression to construct a mouse model of RAS activation. These ACE 8/8 mice die prematurely and abruptly. Here, we have investigated cardiac electrophysiological abnormalities that may contribute to early mortality in this model. In ACE 8/8 mice, surface ECG voltages are reduced. Intracardiac electrograms showed atrial and ventricular potential amplitudes of 11% and 24% compared with matched wild-type (WT) controls. The atrioventricular (AV), atrio-Hisian (AH), and Hisian-ventricular (HV) intervals were prolonged 2.8-, 2.6-, and 3.9-fold, respectively, in ACE 8/8 vs. WT mice. Various degrees of AV nodal block were present only in ACE 8/8 mice. Intracardiac electrophysiology studies demonstrated that WT and heterozygote (HZ) mice were noninducible, whereas 83% of ACE 8/8 mice demonstrated ventricular tachycardia with burst pacing. Atrial connexin 40 (Cx40) and connexin 43 (Cx43) protein levels, ventricular Cx43 protein level, atrial and ventricular Cx40 mRNA abundances, ventricular Cx43 mRNA abundance, and atrial and ventricular cardiac Na⁺ channel (Scn5a) mRNA abundances were reduced in ACE 8/8 compared with WT mice. ACE 8/8 mice demonstrated ventricular Cx43 dephosphorylation. Atrial and ventricular L-type Ca²⁺ channel, Kv4.2 K⁺ channel α -subunit, and Cx45 mRNA abundances and the peak ventricular Na⁺ current did not differ between the groups. In isolated heart preparations, a connexin blocker, 1-heptanol (0.5 mM), produced an electrophysiological phenotype similar to that seen in ACE 8/8 mice. Therefore, cardiac-specific ACE overexpression resulted in changes in connexins consistent with the phenotype of low-voltage electrical activity, conduction defects, and induced ventricular arrhythmia. These results may help explain the increased risk of arrhythmia in states of RAS activation such as heart failure.

Keywords

peptidyl-dipeptidase A; angiotensin II; heart block

Address for reprint requests and other correspondence: S. C. Dudley, Jr., Emory Univ./Atlanta VA Medical Center, 1670 Clairmont Rd. (111B), Decatur, GA 30033 (sdudley@emory.edu).

*V. S. Kasi and H. D. Xiao contributed equally to this work.

Arrhythmic sudden death is a common terminal event in various cardiomyopathies and end-stage heart failure. Upregulation of the renin-angiotensin system (RAS) has been implicated in risk of sudden death in these conditions. A critical component of this system is angiotensin-converting enzyme (ACE), which produces the eight-amino acid peptide angiotensin II (ANG II), a major effector peptide of the RAS. In humans, increased ANG II levels are associated with an increased risk of arrhythmia (2), which is reduced by use of ACE inhibitors or ANG II receptor blockers (4, 13, 20, 23, 27, 30, 49).

A number of ion-handling protein changes have been posited to underlie the increase in risk of arrhythmia in states of RAS activation, and ANG II is known to act on a number of these proteins (3, 41). For example, ANG II has been implicated in Na^+ - K^+ pump regulation (24). Furthermore, ANG II inhibits the Ca^{2+} -activated K^+ current in vascular smooth muscle cells (51) and the delayed rectifier K^+ currents in heart and smooth muscle (11). ANG II has been shown to alter cardiac Cl^- conductance and the transient outward current (I_{to}) (17, 39, 43, 60, 61).

Connexins are transmembrane proteins that assemble as dodecamers and form low-resistance conductive pathways between cardiac myocytes known as gap junctions. The three most prominent cardiac connexins are connexin 40 (Cx40), connexin 43 (Cx43), and connexin 45 (Cx45). In the adult mouse heart, Cx43 is ubiquitously expressed. Cx40 expression is restricted to the atrial myocytes and ventricular conduction system, and Cx45 is weakly expressed in the specialized conduction system and the atrioventricular (AV) node (26, 53). Elimination of any of these connexins alters electrical conduction, increasing risk of arrhythmia (10, 12, 16, 22, 38, 40, 48, 52), and connexin changes have been noted in heart failure (1, 2). ANG II is associated with activation of kinases and phosphatases, and connexin amounts, distribution, and function can be modulated by their phosphorylation state (8, 28).

Recently, we described a cardiac-restricted ACE-overexpression mouse with elevated cardiac ANG II levels and an increased risk of death in the absence of heart failure, significant cellular hypertrophy, or ventricular fibrosis (59). One prominent feature of this ACE 8/8 mouse is a reduction in the surface ECG voltage, without evidence of heart failure, pulmonary edema, or pericardial effusion, suggesting an underlying defect in cardiac current generation or conduction. Therefore, although the reason for the absence of heart failure, hypertrophy, or fibrosis is unknown and may be related to the lack of change in blood pressure in ACE 8/8 mice, this mouse model presented an opportunity to study RAS-mediated electrophysiological abnormalities in the absence of substantial structural changes.

Surface and intracardiac recordings demonstrated reduced voltage, prolonged atrial and ventricular conduction intervals, and various forms of AV nodal block. Ventricular pacing caused ventricular tachycardia only in the ACE 8/8 mice. ECG telemetry identified the cause of death as progressive bradycardia or ventricular tachycardia. Similar phenotypes have been reported in genetically altered mice with reductions in Na^+ channels or connexins (10, 12, 16, 22, 38, 40, 48, 52). In our case, the arrhythmic risk was best explained by changes in gap junction proteins responsible for cell coupling, such as Cx40 and Cx43.

MATERIALS AND METHODS

Cardiac-restricted ACE-overexpression mice

The generation of chimeric mutant ACE mice is described elsewhere (59). Briefly, by gene targeting, the promoter region of the somatic ACE gene was substituted by the α -myosin heavy chain promoter, resulting in cardiac-restricted ACE overexpression. All studies were performed on F₂ generation litters produced by breeding heterozygous mice to create F₂

offspring with wild-type (WT), heterozygous (HZ), and homozygous cardiac-restricted ACE (8/8) overexpression. These mice had a mixed background of C57BL/6 and 129. Male and female mice were used in the studies, with littermate controls matched for age and sex. Genomic DNA was obtained through tail clipping for genotyping, as previously described. Animal procedures were approved by the Institutional Animal Care and Use Committees of Emory University and the Atlanta Veterans Administration.

Surface and telemetry ECG monitoring

After mice were sedated with an intraperitoneal injection of ketamine (125 mg/kg) and xylazine (12.5 mg/kg), their hair was shaved and then completely removed with depilatory (Nair, Church & Dwight, Princeton, NJ). Body temperature was maintained at 37°C with a warm pad. Adhesive skin electrodes were applied to all four limbs and on the anterior (V1) and lateral (V6) chest wall. ECG was recorded digitally for 5 min after 15 min of partial recovery from the anesthesia-induced bradycardia. ECG data were analyzed and stored on a Prucka system (GE Healthcare, Milwaukee, WI).

ECG recordings of awake, free-moving mice were obtained using a telemetry method, as previously described (59). After mice were sedated with ketamine and xylazine (see above), an ECG transmitter (model EA-F20, Data Sciences) was implanted in the intraperitoneal cavity. The positive lead of the transmitter was tunneled subcutaneously into the left anterior chest wall above the apex of the heart and the negative lead to the right shoulder. This configuration approximates lead II on the surface ECG. The ECG was recorded digitally using a 500-Hz analog-to-digital converter and processed with a 100-Hz low-pass filter to reduce noise artifact.

Intracardiac electrophysiology

Intracardiac electrophysiological studies were performed as described elsewhere (9, 10). Body temperature was maintained throughout the procedure with a warming pad placed beneath the animal. A Prucka system (Cathlab 4.0, GE) was used with an Fr-1.1 eight-electrode catheter (model EPR800, Millar Instruments, Houston, TX). The catheter tip was positioned in the right ventricle through a right jugular vein cutdown. ECG channels and intracardiac electrograms were amplified and filtered from 0.05 to 100 Hz and from 30 to 500 Hz, respectively. The sampling rate was 1,024 samples/s, with an accuracy of 12 bits. Atrial and ventricular wave amplitudes were measured over right atrial, His bundle, and right ventricular intracardiac traces and averaged to obtain atrial and ventricular potentials. AV, atrio-Hisian (AH), and His-ventricular (HV) intervals were measured directly from the intracardiac electrogram. Pacing of ventricles was performed at twice the threshold using a Grass SD9 stimulator (Astro-Med, W. Warwick, RI). For induction of ventricular tachycardia, 10–15 stimuli at incrementally decreasing cycle lengths from 150 to 50 ms were applied. Cycle lengths <50 ms could result in nonspecific arrhythmia induction and were not attempted. Inducible tachycardia was defined as more than five consecutive beats (10).

Quantitative mRNA assay

The mRNA abundances of the L-type Ca^{2+} channel (Cacna1), the α -subunit of the voltage-gated $\text{Kv}4.2 \text{ K}^+$ channel (Kcnd2), the cardiac Na^+ channel (Scn5a), and the cardiac connexins Cx40, Cx43, and Cx45 were determined by quantitative real-time RT-PCR. Total RNA from WT, HZ, and ACE 8/8 mice was isolated using Trizol reagent (Invitrogen, Carlsbad, CA) with the addition of RNase-free DNase I following the manufacturer's instructions. RT was carried out at 42°C for 30 min with 1.0 μg of total RNA and the iScript reverse transcriptase (Bio-Rad, Hercules, CA). The first-strand cDNA was used as template for subsequent SYBR real-time PCR. Each PCR contained 12.5 μl of QuantiTect SYBR

Green PCR Kit Master Mix (Bio-Rad) and 2.5 μ M primer pairs of each gene in a total reaction volume of 25 μ L. (See supplemental information in the online version of this article for primers used in the experiment.) All amplifications were performed in triplicate and consisted of 40 cycles of 30 s at 95°C, 30 s at 60°C, and 20 s at 72°C in a thermocycler (iCycler, Bio-Rad). β -Actin was used as an internal reference for quantitative comparisons.

Western blotting and immunohistochemistry

For Cx40 and Cx43 Western blotting, protein samples prepared from atrial and ventricular tissues were homogenized in a buffer containing 20 mM Tris·HCl (pH 7.4), 150 mM NaCl, 2.5 mM EDTA, 1% Triton X-100, 0.5% deoxycholate, 0.1% SDS, 10% glycerol, 50 mM NaF, 1 mM Na_3VO_4 , and 10 μ L/ml protein inhibitor cocktail (Pierce, Rockford, IL). Protein samples (20–100 μ g) were separated on 10% SDS gels and transferred to nitrocellulose membranes. The membranes were blotted with a 1:250 dilution of anti-Cx43 (Zymed, S. San Francisco, CA) or a 1:250 dilution of anti-Cx40 (ADI, San Antonio, TX) antibodies. For Na^+ channel blotting, 100 μ g of protein samples were separated on 6.5% gels. A rabbit anti-pan Na^+ channel antibody was kindly provided by Dr. Peter Mohler (University of Iowa) and used at a 1:1,000 dilution. An anti-GAPDH antibody (1:1,000 dilution; Santa Cruz Biotechnology, Santa Cruz, CA) was used as a loading control for all studies. Donkey anti-rabbit antibody (Amersham, Piscataway, NJ) was used at a dilution 1:10,000. The membranes were exposed to X-ray film using the enhanced chemiluminescence method. The X-ray film images were scanned and analyzed using NIH ImageJ software.

For immunohistochemistry, heart samples were fixed in 10% formalin for 24 h and embedded in paraffin. Heart tissues from ACE 8/8 and WT mice were embedded side-by-side in the paraffin blocks to ensure the identical immunohistochemistry conditions. Tissue sections were reacted with mouse anti-Cx43 antibody (1:500 dilution; BD Bioscience) and then with biotinylated anti-mouse antibody (1: 1,000 dilution; Vector Laboratories, Burlingame, CA) as the secondary antibody. Signals were detected using the Vectastain ABC kit (Vector Laboratories) and diaminobenzidine (DAB+) (brown) chromogen (DAKO, Carpinteria, CA). Tissue sections were then counterstained with aqueous hematoxylin.

Perfused isolated heart preparation

Perfused isolated hearts were prepared as described elsewhere (18). After induction of anesthesia with ketamine-xylazine, hearts were extracted and placed in ice-cold Tyrode solution [in mM: 130 NaCl, 5.4 KCl, 1.0 MgCl_2 , 1.0 CaCl_2 , 0.6 NaHPO_4 , 10.0 HEPES, and 5.0 glucose (pH 7.4)]. Aortas were isolated and cannulated, and the hearts were mounted on a Langendorff apparatus. Isolated hearts were perfused with Tyrode solution at 2 ml/min at 37°C. A pseudo-bipolar ECG was recorded using two silver-silver chloride electrodes placed in the solution bathing the heart. The ECG signals were filtered at 5–400 Hz, sampled at 1,000 Hz, and digitized with 12-bit resolution. After 15 min of perfusion, baseline ECGs were recorded, and the solution was switched to Tyrode solution containing 0.5 mM 1-heptanol, which was washed out, and the heart was reexposed to 1 mM 1-heptanol, which again was washed out. An automated peak-detection algorithm was used to calculate the QRS amplitude for each beat.

Cardiac myocyte isolation

Ventricular cardiac cells were isolated from 6- to 8-wk-old heparinized mice. After the mice were anesthetized with ketamine (100 mg/kg) and xylazine (5 mg/kg), the hearts were removed and rapidly mounted on a Langendorff apparatus. The hearts were perfused with oxygenated Tyrode solution [in mM: 130 NaCl, 5.4 KCl, 1 CaCl_2 , 1 MgCl_2 , 0.6 NaH_2PO_4 , 10 HEPES, and 5 glucose (pH 7.4)] at 37°C. After 5 min, the perfusion solution was replaced with Ca^{2+} -free Tyrode solution for 15 min and then switched to a Tyrode solution

containing type II collagenase (73.7 U/ml; Worthington), 0.1% BSA, 20 mM taurine, and 30 μ M CaCl_2 . After 15 min of enzymatic digestion, the hearts were perfused with Kraft-Brühe solution [100 mM potassium glutamate, 10 mM potassium aspartate, 25 mM KCl, 10 mM KH_2PO_4 , 2 mM MgSO_4 , 20 mM taurine, 5 mM creatinine base, 0.5 mM EGTA, 5 mM HEPES, 1% BSA, and 20 mM glucose (pH 7.2)] for 5 min. Finally, both ventricles were minced into small pieces and gently triturated with a Pasteur pipette for 15–20 min. The cells were collected after centrifugation at 60 *g* for 5 min and stored at 4°C.

Patch clamping

Na^+ current was measured in whole cell voltage-clamp mode with use of an Axopatch 200 B patch-clamp amplifier and a Digidata 1320 Interface (Axon Instruments, Burlingame, CA). pCLAMP software (version 8.0, Axon Instruments) was used for generation of protocols, data storage, and evaluation. Isolated cardiac myocytes were placed in a chamber, mounted on an inverted microscope, and perfused with a bath solution (in mM: 5 NaCl, 1.2 MgCl_2 , 1.8 CaCl_2 , 25 tetraethylammonium chloride, 100 *N*-methyl-D-glucamine, 5 CsCl, 20 HEPES, 11 glucose, 3 4-aminopyridine, and 2 MnCl_2 , with pH adjusted to 7.4 with CsOH). Thin-walled borosilicate patch pipettes (World Precision Instruments, Sarasota, FL) were drawn to a resistance of 1–3 M Ω and filled with a solution containing (in mM) 100 CsCl, 40 CsOH, 10 HEPES, 10 EGTA, 1 CaCl_2 , and 5 Mg-ATP (pH 7.25). Rod-shaped cells with clear striation were selected, and, after a gigaohm seal was established and the membrane patch was ruptured, cell capacitance, access resistance, and membrane resistance were measured. Cells with >10-M Ω access resistance, <100-M Ω membrane resistance, or unstable parameters were excluded. For the purpose of voltage control, in addition to using a low- Na^+ bath solution, we performed the experiments at room temperature and 60–80% compensation for the series resistance (<4-M Ω residual series resistance). Data were filtered at 2 kHz and recorded at 10 kHz. Electronic capacitance compensation was used to minimize current transients during applied pulses. Passive membrane properties were calculated from the current response at –100 to –70 mV and were subtracted from the recorded currents. For determination of steady-state activation, we used an initial holding potential of –100 mV for 200 ms before stepping to –95 to +45 mV in 5-mV increments. For determination of steady-state inactivation kinetics, we held the membrane potential at –100 to +45 mV in 5-mV increments for 200 ms before stepping to –20 mV.

For the measurement of action potentials and resting membrane potential in the current-clamp mode, the bath solution was Tyrode solution (see above) at 37°C, and the pipette solution consisted of (in mM) 140 KCl, 10 HEPES, 10 EGTA, 1 CaCl_2 , and 5 Mg-ATP (pH 7.25). A liquid junction potential of 5 mV was estimated and subtracted from the measured transmembrane potentials.

Statistical analysis

All continuous variables were compared between ACE 8/8 mice, HZ mice, and gender- and age-matched litter-mate controls. Values are means $\pm$ SE. The χ^2 test, Student's *t*-test, one-way ANOVAs with post hoc tests of significance, and linear regression analysis were used where appropriate, and *P* < 0.05 was considered statistically significant.

RESULTS

Surface and telemetry ECG abnormalities in ACE 8/8 mice

Surface ECGs were obtained from anesthetized 6- to 8-wk-old WT, HZ, and ACE 8/8 mice (*n* = 9 in each group). Representative recordings from WT, HZ, and ACE 8/8 mice are shown in Fig. 1. Limb lead I and II traces are shown in Fig. 1A. All ACE 8/8 mice demonstrated no obvious P waves, low-amplitude QRS complexes, and irregular R-R

intervals. Previously, we had interpreted this to imply that the mice were in atrial fibrillation (59). QRS configuration and frontal plane axis were similar in ACE 8/8 and WT mice, suggesting that the difference in voltage was not the result of a QRS axis shift. Moreover, no pericardial or pleural effusions were detected by ultrasound to explain the reduced QRS amplitude.

Intracardiac recordings

To further investigate these surface ECG findings, intracardiac electrograms were obtained from anesthetized 6- to 8-wk-old WT, HZ, and ACE 8/8 mice using a 1.1-Fr, eight-electrode catheter placed transvenously, such that electrodes bridged the right atrium and ventricle. ACE 8/8 animals showed fractionated, low-voltage atrial (a) electrograms, and reduced-voltage ventricular electrograms (Fig. 1B). The maximum voltages for the atrial and ventricular wave electrograms are compared in Table 1. ACE 8/8 mice showed a reduction in atrial and ventricular potential amplitudes of 9.0- and 4.2-fold, respectively, relative to WT mice ($P < 0.01$ for each potential). These changes were on the order of those seen on the surface ECG, consistent with a cardiac cause of the reduced surface voltage. There was a trend toward a graded reduction in intracardiac electrogram voltage as a function of ACE gene dose, with effects more prominent in the atria than ventricles. Nevertheless, the difference between WT and HZ mice was not statistically significant.

Abnormalities in the intracardiac electrograms were associated with conduction delays. AV, AH, and HV intervals were prolonged in the ACE 8/8 mice ($P < 0.01$), suggesting conduction slowing at multiple cardiac levels (Table 1). These prolongations remained significant when corrected for heart rate differences among the groups. In addition to conduction delays, ACE 8/8 mice showed various degrees of AV block compared with WT or HZ mice. Two, 3, and 6 of the 11 ACE 8/8 mice exhibited first-, second-, and third-degree AV nodal heart block, respectively. Although some WT mouse strains are known to show intermittent heart block (32), in our studies, only the ACE 8/8 mice demonstrated this arrhythmia.

Tachyarrhythmic susceptibility

Slow conduction is known to increase the risk of ventricular tachyarrhythmias by reducing the reentry wavelength (defined as $\lambda = CV \times ERP$, where CV is conduction velocity and ERP is effective refractory period). Electrical burst pacing was used to correlate ventricular arrhythmic risk with ACE expression. Ventricular pacing demonstrated no proclivity toward induced ventricular tachyarrhythmias in WT and HZ mice ($n = 7$ for each group). On the other hand, five of six ACE 8/8 mice showed inducible ventricular tachycardia on ventricular pacing ($P < 0.01$). An example of tachyarrhythmia induction in an ACE 8/8 mouse is shown in Fig. 2A.

ECG telemetry revealed the cause of death in ACE 8/8 mice to be a sudden loss of the ventricular escape rhythm in the presence of AV block or ventricular tachyarrhythmia. An example of an ACE 8/8 mouse developing fatal, sudden-onset ventricular tachycardia is shown in Fig. 2B.

Connexin and Na⁺ channel protein expression

The electro-physiological findings in ACE 8/8 mice suggested a defect in channels responsible for cardiac current conduction. In addition, the electrophysiological findings in ACE 8/8 mice were similar to those in mice with downregulation of cardiac connexins or the cardiac Na⁺ channel (10, 12, 16, 22, 38, 40, 48, 52). Under the usual physiological conditions in cardiac muscle, the Na⁺ channel generates the largest current, and connexins forming gap junction channels provide the critical, low-resistance pathway between cells for

conduction. Since previously reported microscopic analysis showed minimal fibrosis or ultrastructural changes in the ACE 8/8 ventricles (59), we investigated whether some of the changes in conduction could be explained by alterations in these ion channels.

Cx40 is expressed mostly in the atrium (26, 53). In this chamber, Western analysis showed reductions in Cx40 (Fig. 3, A and B). Atrial Cx43 protein showed a graded reduction with ACE gene dose (Fig. 3, C and D). In the ventricles, Cx43 was downregulated in the ACE 8/8 mice to 30% of WT levels (Fig. 3, E and F). This downregulation was confirmed by immunohistochemistry, which showed a marked decrease of Cx43 staining in the ventricles of ACE 8/8 mice (Fig. 3H) compared with WT mice (Fig. 3G). Similar to the WT mice, the majority of Cx43 expression in ACE 8/8 hearts was located at the intercalated disks. Another major channel affecting conduction is the cardiac Na⁺ channel (31, 36, 40). Western analysis showed no changes in atrial or ventricular Na⁺ channel levels, however (data not shown).

Cx43 has multiple phosphorylated forms with different electrophoretic mobilities (35). The nonphosphorylated form (P0) usually migrates as a single band to 41 kDa, whereas the phosphorylated forms (P1, P2, and P3) migrate to 42–46 kDa. The nonphosphorylated P0 form is thought not to conduct (6). Therefore, we investigated whether changes in Cx43 phosphorylation played a role in the ACE mouse phenotype. Figure 4 shows an association between the surface ECG voltage amplitude and Cx43 expression across genotypes. Nevertheless, total ventricular Cx43 was only weakly associated with the QRS amplitude ($r^2 = 0.19$). A reduction in the ratio of phosphorylated to nonphosphorylated Cx43 [(P1 + P2 + P3)/P0] as a function of genotype (Fig. 4B), which correlated more strongly with the ECG amplitude (Fig. 4C), better explained the changes in QRS amplitude.

To study changes in the Na⁺ channel, we measured Na⁺ current using the patch-clamp technique. The resting membrane potential was similar between WT- and ACE 8/8 mice: -67 ± 2.1 and -72 ± 1.3 mV, respectively ($n = 5$ in each group, $P = 0.08$). The membrane capacitances were 145.1 ± 10.1 pF in WT mice and 120.7 ± 8.1 pF in ACE 8/8 mice ($n = 14$ for each group, $P = 0.07$), suggesting no cellular hypertrophy in ACE 8/8 mice. Na⁺ current was compared between WT and ACE 8/8-derived ventricular myocytes. There was no significant difference in the peak Na⁺ conductance between WT and ACE 8/8 mice when a total of 10 cells from each group were measured ($n = 3$ mice from each group; Fig. 5A), but the activation and inactivation curves were shifted toward more negative values in ACE 8/8 mice (Fig. 5, B and C).

Connexin transcriptional regulation

Transcriptional regulation has been implicated in ion channel changes during a number of cardiac pathogenic states associated with increased arrhythmic risk (3, 41). To determine whether transcriptional regulation could explain some of the changes, connexin and Na⁺ channel mRNA abundance levels were examined by quantitative RT-PCR. The L-type Ca²⁺ channel (Cacna1) and I_{to}, encoded in part by Kv4.2 (Kcnd2), were studied also because they are prominent ion channels that have been reported to be dynamically regulated under various pathogenic conditions (5, 29, 50, 54–56, 58).

Cardiac ACE overexpression was associated with reductions in Cx40 mRNA (Fig. 6A) in the atria and ventricles of ACE 8/8 mice ($n = 4$) relative to WT mice ($n = 4$, $P < 0.05$) and an elimination of the AV difference in Cx40 mRNA abundances in WT mice. Graded reductions of Cx40 mRNA were also noted in HZ mice ($n = 4$) relative to WT mice ($P < 0.05$). Cx43 mRNA levels were reduced only in the atria, however (Fig. 6B; $P < 0.05$). mRNA abundances for Cx45 (Fig. 6C), an I_{to} α -subunit (Kcnd2; Fig. 6D), and the L-type Ca²⁺ channel (Cacna1; Fig. 6E) were unchanged in the atria or ventricles of ACE 8/8

compared with WT mice. Despite the lack of significant change in the peak Na⁺ channel current, Scn5a mRNA abundances showed a significant drop in atria and ventricles of ACE 8/8 mice ($n = 4$) relative to WT mice ($n = 4$, $P < 0.05$; Fig. 6F).

A connexin blocker produced a similar electrophysiological phenotype

As mentioned above, a reduction in QRS amplitude (surface and intracardiac) is one of the hallmarks of ACE 8/8 mice. Although decreases in Cx40 and Cx43 could explain the conduction abnormalities in these mice, it is not clear that changes in connexins were responsible for the low-voltage electrical activity. With the use of a perfused isolated heart model and WT mouse hearts, perfusion with the gap junction blocker 1-heptanol, at ≤ 1.0 mM, resulted in a reduced relative QRS amplitude of 0.5 ± 0.1 ($n = 5$, $P = 0.001$) without a significant change in heart rate, QRS width, or P-R interval (Fig. 7). Although the mechanism for the voltage reduction in mice is unclear, it is conceivable that slow conduction resulting from reduced gap junctional conductance increases the overlap of depolarization and repolarization events in the mouse heart, allowing these two electrically opposite vectors to show a higher degree of cancellation.

DISCUSSION

Previously, we reported that cardiac-restricted ACE overexpression in a mouse resulted in abnormalities of the surface ECG and sudden death in the absence of significant ventricular structural abnormalities, hypertrophy, fibrosis, or heart failure (59). Here, we investigate further the origins of those abnormalities. Compared with WT controls, ACE 8/8 mice showed slowed conduction at multiple levels, various degrees of AV nodal block, and inducible ventricular arrhythmias.

On the basis of the absence of P waves and an irregularly irregular QRS rhythm on the surface ECG, we suggested previously that ACE 8/8 mice had atrial fibrillation (59). Nevertheless, the intracardiac electrograms did not show this to be the case in the ACE 8/8 mice. In all cases, these mice had low-voltage, fractionated atrial electrograms that were not detectable on the surface ECG, and, when combined with variable AV block, the surface ECG changes were indistinguishable from atrial fibrillation. Although we did not observe atrial fibrillation during intracardiac recordings in our study of relatively young ACE 8/8 mice, the abnormal atrial electro-physiology would suggest the risk for this arrhythmia, and we cannot completely exclude the possibility of such an occurrence at some point in these mice.

Downregulation of connexins in ACE 8/8 mice may be the major mediators of conduction defects

These changes were associated with alterations of connexins, the constituents of gap junctions, which are known to be important in conduction. These alterations included transcriptional downregulation of atrial and ventricular Cx40 and ventricular Cx43, reductions in atrial Cx40 and atrial and ventricular Cx43 protein, and Cx43 dephosphorylation. ACE 8/8 mice showed evidence of atrial and ventricular slow conduction and AV block consistent with that previously seen with the combination of downregulation of Cx40 and Cx43. Cx40 deficiency results in atrial, AV nodal, and infra-Hisian conduction block (10, 48, 52). Similar conduction defects were observed in the ACE 8/8 mice in association with a downregulation of Cx40. Cx40-null mice do not show changes in alterations in surface QRS voltage or ventricular tachyarrhythmia inducibility (57), suggesting that additional factors are involved in producing the ACE 8/8 phenotype. The susceptibility of ACE 8/8 mice to ventricular arrhythmias is more consistent with a

reduction of Cx43, since mouse models of reduced Cx43 show prominent ventricular arrhythmias (12, 16, 22).

The idea that Cx40 and Cx43 changes were mainly responsible for the ACE 8/8 phenotype is supported by the absence of statistical changes in Na⁺ channel total protein or current or transcriptional regulation of the L-type Ca²⁺ or the Kv4.2 channels. Also, the ECG features of the ACE 8/8 mice were similar to those induced by a gap junction blocker, heptanol. At ≤1 mM, heptanol is thought to be a fairly specific blocker of gap junctions, although it can inhibit Na⁺ channels at higher concentrations (37, 47).

In humans, it is well established that downregulation of the cardiac Na⁺ channel can be associated with conduction abnormalities and ventricular tachycardia (31, 36). Disruption of the mouse cardiac Na⁺ channel gene causes AV conduction blocks, delayed intramyocardial conduction, increased ventricular refractoriness, and ventricular tachycardia (40). Nevertheless, in the ACE 8/8 mice, we saw significant reductions in mRNA abundance, but peak current was not statistically different from that in WT mice. These data suggest that RAS activation altered Na⁺ channel transcription but that there was posttranscriptional compensation in our model. Examples of posttranscriptional Na⁺ channel modulation include protein kinase A-mediated phosphorylation effects that can enhance channel current (62), and gating changes similar to those we observed have been noted with protein kinase A and C activation (34, 42). Among the possible explanations for the discrepancy between mRNA abundance and current are increased translational efficiency and reduced channel degradation. Also, although peak Na⁺ current was unchanged between the two groups, on the basis of the differences in resting membrane potentials and the steady-state inactivation curves, the Na⁺ channel availability is estimated to be 79% in WT mice and 66% in ACE 8/8 mice. Therefore, we cannot rule out completely that a reduction in Na⁺ channel current plays some role in the slow conduction phenotype of the ACE 8/8 mice.

Other channels studied by RT-PCR, including the cardiac Na⁺ channel, the L-type Ca²⁺ channel, Kv4.2, and Cx45, did not show changes that would explain the phenotype. We cannot rule out that posttranscriptional changes play some role, however. This result is consistent with a previous report about the effects of ANG II on *I*_{to} (60). Also, the similarity in resting membrane potential between the WT and ACE 8/8 groups suggests that significant RAS-mediated changes in the Na⁺-K⁺ pump, inwardly rectifying K⁺ current, or other factors setting the resting membrane potential are unlikely to contribute to the phenotype. On the basis of the expression pattern of Cx45 in the heart and the presence of AV block in the ACE 8/8 mice, it was unexpected that Cx45 mRNA levels were unchanged, however. Although changes in Cx40 and Cx43 are sufficient to explain the blocks, we cannot rule out that Cx45 changes play a role, since posttranscriptional alterations and changes localized to the AV node were not excluded in our study. In summary, although we have not excluded all other possible causes of the electrophysiological phenotype resulting from cardiac ACE overexpression, the phenotype is consistent with the Cx40 and Cx43 changes noted.

Why did HZ ACE mice not show increased arrhythmias?

The ACE 8/8 mouse phenotype is consistent with manifestations of combined Cx40 and Cx43 reductions. The HZ mice showed intermediate reductions of ion channels and a phenotype most similar to the WT mice. These findings are consistent with those in Cx40 mice heterozygous for their null mutation (57). The finding that mice bred to have gradual reductions in Cx43 do not show ventricular tachyarrhythmia inducibility until the connexin level falls to less than ~40% of normal suggests a threshold effect of reduction in gap junctional conductance on electrophysiological manifestations (i.e., the safety factor) (12). The idea of a threshold for phenotypic manifestations is supported by theoretical calculations (46).

Possible mediators of the ACE effect

Although we have not established the cause of the molecular changes noted, it is likely an effect of local ACE expression, since previous gene-targeted constructs overexpressing ACE in other organs (such as liver-restricted ACE overexpression) do not show electrical abnormalities similar to the ACE 8/8 mice (7). Given the well-known effect of ACE to produce ANG II and the elevated cardiac ANG II levels of the ACE 8/8 mice, it is tempting to speculate that the effect of cardiac ACE overexpression on arrhythmic risk is the product of ANG II elevation.

Our results suggest that RAS activation can affect connexin transcriptional and posttranscriptional regulation. These results are consistent with recent reports of Cx43 downregulation and dephosphorylation in heart failure (1, 2). ANG II is known to affect transcription of multiple genes (45), possibly explaining the mRNA changes. Nevertheless, the origin of the chamber-specific regulation of connexins is unclear. The AV differences in Cx40 mRNA abundance in WT mice were eliminated in ACE 8/8 mice, but Cx43 changes were better explained by posttranscriptional mechanisms, especially in the ventricle. Connexin turnover and activity are influenced by a number of kinases and phosphatases (8, 28). Increased phosphorylation and dephosphorylation have been associated with decreased gap junctional conductance. In our study, conduction impairment was associated with Cx43 dephosphorylation. A similar association was reported recently in human heart failure and appeared to be explained by increased association between Cx43 and the PP2A phosphatase (1). Interestingly, ANG II is known to activate this phosphatase (25). Nevertheless, functional gap junctions are multiprotein complexes, and RAS effects on connexin-associated proteins cannot be ruled out as a mechanism for the observed changes (21, 44).

On the other hand, the situation may be more complicated. ACE2 is an ACE homolog expressed in many tissues, including the heart, which is thought to help counterbalance ACE in regulating ANG II levels by proteolytically cleaving a single amino acid from ANG I and ANG II, producing ANG-(1–9) and ANG-(1–7), respectively.(14) Cardiac ACE2 overexpression results in a phenotype of reduced connexins, conduction defects, and arrhythmic risk remarkably similar to that in our ACE 8/8 mice (15). Other possible mediators include alternative substrates for ACE and signaling mediated by the ACE COOH terminus (19). Although the exact mediator of the electrophysiological changes has yet to be determined, it seems clear that alterations in cardiac ACE activity and the resulting abnormalities of RAS have profound effects on cardiac electrophysiology.

In summary, we found that cardiac ACE overexpression is associated with connexin dysregulation that is correlated with conduction defects. This is the first report of ACE expression affecting connexin regulation. If RAS activation in humans were to be associated with similar changes in ACE expression as seen in our ACE 8/8 model, this would help explain the arrhythmic risk seen in these conditions (33). Furthermore, this finding may help explain the antiarrhythmic effects of ANG II receptor blockers (20) and ACE inhibitors (4, 13, 23, 27, 49).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

GRANTS

This study was supported by National Institutes of Health Grants HL-64828, DK-039777, DK-051445, and DK-055503, a Department of Veterans Affairs merit grant (S. C. Dudley, Jr.), an American Heart Association

Established Investigator Award (S. C. Dudley, Jr.), and National Institutes of Health Research Fellowship National Research Service Award F32 (V. S. Kasi). H. D. Xiao is supported by an American Heart Association Scientist Development Grant.

References

1. Ai X, Pogwizd SM. Connexin 43 downregulation and dephosphorylation in nonischemic heart failure is associated with enhanced colocalized protein phosphatase type 2A. *Circ Res.* 2005; 96:54–63. [PubMed: 15576650]
2. Akar FG, Spragg DD, Tunin RS, Kass DA, Tomaselli GF. Mechanisms underlying conduction slowing and arrhythmogenesis in nonischemic dilated cardiomyopathy. *Circ Res.* 2004; 95:717–725. [PubMed: 15345654]
3. Akar FG, Tomaselli GF. Ion channels as novel therapeutic targets in heart failure. *Ann Med.* 2005; 37:44–54. [PubMed: 15902846]
4. Ambrosioni E, Borghi C, Magnani B. The effect of the angiotensin-converting enzyme inhibitor zofenopril on mortality and morbidity after anterior myocardial infarction. The Survival of Myocardial Infarction Long-Term Evaluation (SMILE) Study Investigators. *N Engl J Med.* 1995; 332:80–85. [PubMed: 7990904]
5. Aroundas AA, Wu R, Juang G, Marban E, Tomaselli GF. Electrical and structural remodeling of the failing ventricle. *Pharmacol Ther.* 2001; 92:213–230. [PubMed: 11916538]
6. Beardslee MA, Lerner DL, Tadros PN, Laing JG, Beyer EC, Yamada KA, Kleber AG, Schuessler RB, Saffitz JE. Dephosphorylation and intracellular redistribution of ventricular connexin43 during electrical uncoupling induced by ischemia. *Circ Res.* 2000; 87:656–662. [PubMed: 11029400]
7. Bernstein KE. Views of the renin-angiotensin system: brilling, mimsy, and slithy tove. *Hypertension.* 2006; 47:509–514. [PubMed: 16365193]
8. Berthoud VM, Minogue PJ, Laing JG, Beyer EC. Pathways for degradation of connexins and gap junctions. *Cardiovasc Res.* 2004; 62:256–267. [PubMed: 15094346]
9. Berul CI, Aronovitz MJ, Wang PJ, Mendelsohn ME. In vivo cardiac electrophysiology studies in the mouse. *Circulation.* 1996; 94:2641–2648. [PubMed: 8921812]
10. Bevilacqua LM, Simon AM, Maguire CT, Gehrmann J, Wakimoto H, Paul DL, Berul CI. A targeted disruption in connexin40 leads to distinct atrioventricular conduction defects. *J Interv Card Electrophysiol.* 2000; 4:459–467. [PubMed: 11046183]
11. Daleau P, Turgeon J. Angiotensin II modulates the delayed rectifier potassium current of guinea pig ventricular myocytes. *Pflügers Arch.* 1994; 427:553–555.
12. Danik SB, Liu F, Zhang J, Suk HJ, Morley GE, Fishman GI, Gutstein DE. Modulation of cardiac gap junction expression and arrhythmic susceptibility. *Circ Res.* 2004; 95:1035–1041. [PubMed: 15499029]
13. Domanski MJ, Exner DV, Borkowf CB, Geller NL, Rosenberg Y, Pfeffer MA. Effect of angiotensin-converting enzyme inhibition on sudden cardiac death in patients following acute myocardial infarction. A meta-analysis of randomized clinical trials. *J Am Coll Cardiol.* 1999; 33:598–604. [PubMed: 10080457]
14. Donoghue M, Hsieh F, Baronas E, Godbout K, Gosselin M, Stagliano N, Donovan M, Woolf B, Robison K, Jeyaseelan R, Breitbart RE, Acton S. A novel angiotensin-converting enzyme-related carboxypeptidase (ACE2) converts angiotensin I to angiotensin 1–9. *Circ Res.* 2000; 87:E1–E9. [PubMed: 10969042]
15. Donoghue M, Wakimoto H, Maguire CT, Acton S, Hales P, Stagliano N, Fairchild-Huntress V, Xu J, Lorenz JN, Kadambi V, Berul CI, Breitbart RE. Heart block, ventricular tachycardia, and sudden death in ACE2 transgenic mice with downregulated connexins. *J Mol Cell Cardiol.* 2003; 35:1043–1053. [PubMed: 12967627]
16. Eckardt D, Theis M, Degen J, Ott T, van Rijen HV, Kirchhoff S, Kim JS, de Bakker JM, Willecke K. Functional role of connexin43 gap junction channels in adult mouse heart assessed by inducible gene deletion. *J Mol Cell Cardiol.* 2004; 36:101–110. [PubMed: 14734052]
17. Enos R, Coetzee WA, Opie LH. Effects of the ACE inhibitor, perindoprilat, and of angiotensin II on the transient inward current of guinea pig ventricular myocytes. *J Cardiovasc Pharmacol.* 1992; 19:17–23. [PubMed: 1375683]

18. Fiset C, Clark RB, Larsen TS, Giles WR. A rapidly activating sustained K^+ current modulates repolarization and excitation-contraction coupling in adult mouse ventricle. *J Physiol.* 1997; 504:557–563. [PubMed: 9401964]
19. Fleming I. Signaling by the angiotensin-converting enzyme. *Circ Res.* 2006; 98:887–896. [PubMed: 16614314]
20. Gavras I, Gavras H. The antiarrhythmic potential of angiotensin II antagonism: experience with losartan. *Am J Hypertens.* 2000; 13:512–517. [PubMed: 10826402]
21. Giepmans BN. Gap junctions and connexin-interacting proteins. *Cardiovasc Res.* 2004; 62:233–245. [PubMed: 15094344]
22. Gutstein DE, Morley GE, Tamaddon H, Vaidya D, Schneider MD, Chen J, Chien KR, Stuhlmann H, Fishman GI. Conduction slowing and sudden arrhythmic death in mice with cardiac-restricted inactivation of connexin43. *Circ Res.* 2001; 88:333–339. [PubMed: 11179202]
23. Hattori Y, Atsushi S, Hiroaki F, Toyama J. Effects of cilazapril on ventricular arrhythmia in patients with congestive heart failure. *Clin Ther.* 1997; 19:481–486. [PubMed: 9220212]
24. Hool LC, Gray DF, Robinson BG, Rasmussen HH. Angiotensin-converting enzyme inhibitors regulate the Na^+-K^+ pump via effects on angiotensin metabolism. *Am J Physiol Cell Physiol.* 1996; 271:C172–C180.
25. Huang XC, Sumners C, Richards EM. Angiotensin II stimulates protein phosphatase 2A activity in cultured neuronal cells via type 2 receptors in a pertussis toxin-sensitive fashion. *Adv Exp Med Biol.* 1996; 396:209–215. [PubMed: 8726701]
26. Jalife J, Morley GE, Vaidya D. Connexins and impulse propagation in the mouse heart. *J Cardiovasc Electrophysiol.* 1999; 10:1649–1663. [PubMed: 10636196]
27. Kober L, Torp-Pedersen C, Carlsen JE, Bagger H, Eliassen P, Lyng-borg K, Videbaek J, Cole DS, Auclert L, Pauly NC. A clinical trial of the angiotensin-converting enzyme inhibitor trandolapril in patients with left ventricular dysfunction after myocardial infarction. Trandolapril Cardiac Evaluation (TRACE) Study Group. *N Engl J Med.* 1995; 333:1670–1676. [PubMed: 7477219]
28. Lampe PD, Lau AF. The effects of connexin phosphorylation on gap junctional communication. *Int J Biochem Cell Biol.* 2004; 36:1171–1186. [PubMed: 15109565]
29. Libbus I, Rosenbaum DS. Remodeling of cardiac repolarization: mechanisms and implications of memory. *Card Electrophysiol Rev.* 2002; 6:302–310. [PubMed: 12114856]
30. Lindholm LH, Dahlof B, Edelman JM, Ibsen H, Borch-Johnsen K, Olsen MH, Snapinn S, Wachtell K. Effect of losartan on sudden cardiac death in people with diabetes: data from the LIFE study. *Lancet.* 2003; 362:619–620. [PubMed: 12944063]
31. Liu H, Clancy C, Cormier J, Kass R. Mutations in cardiac sodium channels: clinical implications. *Am J Pharmacogenomics.* 2003; 3:173–179. [PubMed: 12814325]
32. London B. Cardiac arrhythmias: from (transgenic) mice to men. *J Cardiovasc Electrophysiol.* 2001; 12:1089–1091. [PubMed: 11573703]
33. Lonn EM, Yusuf S, Jha P, Montague TJ, Teo KK, Benedict CR, Pitt B. Emerging role of angiotensin-converting enzyme inhibitors in cardiac and vascular protection. *Circulation.* 1994; 90:2056–2069. [PubMed: 7923694]
34. Muramatsu H, Kiyosue T, Arita M, Ishikawa T, Hidaka H. Modification of cardiac sodium current by intracellular application of cAMP. *Pflügers Arch.* 1994; 426:146–154.
35. Musil LS, Cunningham BA, Edelman GM, Goodenough DA. Differential phosphorylation of the gap junction protein connexin43 in junctional communication-competent and -deficient cell lines. *J Cell Biol.* 1990; 111:2077–2088. [PubMed: 2172261]
36. Napolitano C, Rivolta I, Priori SG. Cardiac sodium channel diseases. *Clin Chem Lab Med.* 2003; 41:439–444. [PubMed: 12747584]
37. Nelson WL, Makielski JC. Block of sodium current by heptanol in voltage-clamped canine cardiac Purkinje cells. *Circ Res.* 1991; 68:977–983. [PubMed: 1849060]
38. Nishii K, Kumai M, Egashira K, Miwa T, Hashizume K, Miyano Y, Shibata Y. Mice lacking connexin45 conditionally in cardiac myocytes display embryonic lethality similar to that of germline knockout mice without endocardial cushion defect. *Cell Commun Adhes.* 2003; 10:365–369. [PubMed: 14681043]

39. Obayashi K, Horie M, Xie LH, Tsuchiya K, Kubota A, Ishida H, Sasayama S. Angiotensin II inhibits protein kinase A-dependent chloride conductance in heart via pertussis toxin-sensitive G proteins. *Circulation*. 1997; 95:197–204. [PubMed: 8994437]
40. Papadatos GA, Wallerstein PM, Head CE, Ratcliff R, Brady PA, Benndorf K, Saumarez RC, Trezise AE, Huang CL, Vandenberg JI, Colledge WH, Grace AA. Slowed conduction and ventricular tachycardia after targeted disruption of the cardiac sodium channel gene *Scn5a*. *Proc Natl Acad Sci USA*. 2002; 99:6210–6215. [PubMed: 11972032]
41. Pogwizd SM, Bers DM. Cellular basis of triggered arrhythmias in heart failure. *Trends Cardiovasc Med*. 2004; 14:61–66. [PubMed: 15030791]
42. Qu Y, Rogers JC, Tanada TN, Catterall WA, Scheuer T, Qu Y, Rogers JC, Tanada TN, Catterall WA, Scheuer T. Phosphorylation of S1505 in the cardiac Na⁺ channel inactivation gate is required for modulation by protein kinase C. *J Gen Physiol*. 1996; 108:375–379. [PubMed: 8923263]
43. Ricard P, Danilo P Jr, Cohen IS, Burkhoff D, Rosen MR. A role for the renin-angiotensin system in the evolution of cardiac memory. *J Cardiovasc Electrophysiol*. 1999; 10:545–551. [PubMed: 10355696]
44. Saffitz JE. Adhesion molecules: why they are important to the electro-physiologist. *J Cardiovasc Electrophysiol*. 2006; 17:225–229. [PubMed: 16533266]
45. Schwartz F, Duka A, Duka I, Cui J, Gavras H, Schwartz F, Duka A, Duka I, Cui J, Gavras H. Novel targets of ANG II regulation in mouse heart identified by serial analysis of gene expression. *Am J Physiol Heart Circ Physiol*. 2004; 287:H1957–H1966. [PubMed: 15242839]
46. Shaw RM, Rudy Y. Ionic mechanisms of propagation in cardiac tissue. Roles of the sodium and L-type calcium currents during reduced excitability and decreased gap junction coupling. *Circ Res*. 1997; 81:727–741. [PubMed: 9351447]
47. Takens-Kwak BR, Jongsma HJ, Rook MB, van Ginneken AC. Mechanism of heptanol-induced uncoupling of cardiac gap junctions: a perforated patch-clamp study. *Am J Physiol Cell Physiol*. 1992; 262:C1531–C1538.
48. Tamaddon HS, Vaidya D, Simon AM, Paul DL, Jalife J, Morley GE. High-resolution optical mapping of the right bundle branch in connexin40 knockout mice reveals slow conduction in the specialized conduction system. *Circ Res*. 2000; 87:929–936. [PubMed: 11073890]
49. Teo KK, Mitchell LB, Pogue J, Bosch J, Dagenais G, Yusuf S. Effect of ramipril in reducing sudden deaths and nonfatal cardiac arrests in high-risk individuals without heart failure or left ventricular dysfunction. *Circulation*. 2004; 110:1413–1417. [PubMed: 15353497]
50. Tomaselli GF, Rose J. Molecular aspects of arrhythmias associated with cardiomyopathies. *Curr Opin Cardiol*. 2000; 15:202–208. [PubMed: 10952429]
51. Toro L, Amador M, Stefani E. ANG II inhibits calcium-activated potassium channels from coronary smooth muscle in lipid bilayers. *Am J Physiol Heart Circ Physiol*. 1990; 258:H912–H915.
52. VanderBrink BA, Sellitto C, Saba S, Link MS, Zhu W, Homoud MK, Estes NA 3rd, Paul DL, Wang PJ. Connexin40-deficient mice exhibit atrioventricular nodal and infra-Hisian conduction abnormalities. *J Cardiovasc Electrophysiol*. 2000; 11:1270–1276. [PubMed: 11083248]
53. Van Veen AA, van Rijen HV, Opthof T. Cardiac gap junction channels: modulation of expression and channel properties. *Cardiovasc Res*. 2001; 51:217–229. [PubMed: 11470461]
54. Van Wagoner DR. Pharmacologic relevance of K⁺ channel remodeling in atrial fibrillation. *J Mol Cell Cardiol*. 2000; 32:1763–1766. [PubMed: 11013120]
55. Van Wagoner DR, Nerbonne JM. Molecular basis of electrical remodeling in atrial fibrillation. *J Mol Cell Cardiol*. 2000; 32:1101–1117. [PubMed: 10888261]
56. Van Wagoner DR, Pond AL, Lamorgese M, Rossie SS, McCarthy PM, Nerbonne JM. Atrial L-type Ca²⁺ currents and human atrial fibrillation. *Circ Res*. 1999; 85:428–436. [PubMed: 10473672]
57. Verheule S, van Batenburg CA, Coenjaerts FE, Kirchhoff S, Willecke K, Jongsma HJ. Cardiac conduction abnormalities in mice lacking the gap junction protein connexin40. *J Cardiovasc Electrophysiol*. 1999; 10:1380–1389. [PubMed: 10515563]

58. Wang Z, Kutschke W, Richardson KE, Karimi M, Hill JA. Electrical remodeling in pressure-overload cardiac hypertrophy: role of calcineurin. *Circulation*. 2001; 104:1657–1663. [PubMed: 11581145]
59. Xiao HD, Fuchs S, Campbell DJ, Lewis W, Dudley SC Jr, Kasi VS, Hoit BD, Keshelava G, Zhao H, Capecchi MR, Bernstein KE. Mice with cardiac-restricted angiotensin-converting enzyme (ACE) have atrial enlargement, cardiac arrhythmia and sudden death. *Am J Pathol*. 2004; 165:1019–1032. [PubMed: 15331425]
60. Yu H, Gao J, Wang H, Wymore R, Steinberg S, McKinnon D, Rosen MR, Cohen IS. Effects of the renin-angiotensin system on the current I_{to} in epicardial and endocardial ventricular myocytes from the canine heart. *Circ Res*. 2000; 86:1062–1068. [PubMed: 10827136]
61. Zhang TT, Takimoto K, Stewart AF, Zhu C, Levitan ES. Independent regulation of cardiac Kv4.3 potassium channel expression by angiotensin II and phenylephrine. *Circ Res*. 2001; 88:476–482. [PubMed: 11249870]
62. Zhou J, Shin HG, Yi J, Shen W, Williams CP, Murray KT. Phosphorylation and putative ER retention signals are required for protein kinase A-mediated potentiation of cardiac sodium current. *Circ Res*. 2002; 91:540–546. [PubMed: 12242273]

Fig. 1.

Electrophysiological comparison of 6- to 8-wk-old wild-type (WT) mice, heterozygous (HZ) mice, and a homozygous mouse model of renin-angiotensin system activation that uses cardiac-restricted angiotensin-converting enzyme (ACE 8/8 mice). *A*: representative standard surface ECG limb leads I and II in WT, HZ, and ACE 8/8 mice. QRS amplitude of ACE 8/8 mice was markedly reduced. ACE 8/8 mice ECGs showed no evidence of P waves and irregular R-R intervals. All traces are presented at the same sweep speed and amplification. *B*: representative surface lead II and intracardiac (His lead) recordings from a WT mouse. There are distinct, high-amplitude atrial (A), Hisian (H), and ventricular (V) electrograms. Note low-amplitude and fractionated atrial activity in the ACE 8/8 mouse and prolonged AH and HV intervals compared with the WT mouse. Sweep speed and amplifier gain are the same in both cases.

Fig. 2.

Evidence of arrhythmic risk in ACE 8/8 mice. **A**: ventricular tachyarrhythmic induction by burst pacing. An 18-beat burst of extrastimuli at a 50-ms cycle length was unable to induce ventricular tachycardia in a WT mouse, whereas a 13-beat burst of extra-stimuli at the same cycle length was sufficient to induce polymorphic ventricular tachycardia in an ACE 8/8 mouse. Representative surface ECG leads I and the intracardiac right atrial (RA) and His leads are shown. Only ACE 8/8 mice were inducible. **B**: representative sequential recordings of standard surface ECG limb lead II at the time of death of an ACE 8/8 mouse. *a*: Baseline ventricular rate was 420 min^{-1} , and QRS complexes are indicated by solid arrows. A premature beat (open arrow) initiates ventricular tachycardia at $>1,200 \text{ min}^{-1}$. High-frequency bursts on the ECG, seen most clearly during the initial rhythm, represent diaphragmatic activity. *b*: Continuation of ventricular tachycardia with a “torsades de pointes” morphology. *c*: A more regular ventricular tachycardia that culminates in asystole, as recorded in *d*.

Fig. 3.

Reduced connexin protein expression in ACE 8/8 mice. *A*, *C*, and *E*: Western blot analysis of atrial Cx40 and atrial and ventricular Cx43 expression in WT, HZ, and ACE 8/8 mice. *B*, *D*, and *F*: quantification of results from analysis in *A*, *C*, and *E*, respectively, expressed relative to GAPDH. Values are means $\pm$ SE ($n = 6$). * $P < 0.05$. *G* and *H*: anti-Cx43 antibody-stained sections of cardiac ventricles from WT and ACE 8/8 mice, respectively. Sections are representative of 4 similar results. Cx43 staining is shown as brown pigment. Arrows, Cx43 expression in the intercalated disk regions in WT and ACE 8/8 ventricles. Cx43 expression in ACE 8/8 ventricles remains in the intercalated disks, but expression level is markedly decreased.

Fig. 4.

Relation between Cx43 and surface QRS amplitude in ACE 8/8 mice. **A:** Western analysis of Cx43 expression in the ventricle of WT, HZ, and ACE 8/8 mice as a function of the corresponding QRS complexes (surface lead II). **B:** ratio of phosphorylated (P1 + P2 + P3) Cx43 to the nonphosphorylated form (P0) for each genotype. $*P < 0.05$. **C:** ratio of phosphorylated (P1 + P2 + P3) to nonphosphorylated Cx43 plotted against the QRS amplitude (composite frontal vector calculated from leads I and aVF). QRS amplitude refers to frontal-plane amplitude of the QRS vector, calculated as $\sqrt{I^2 + aVF^2}$.

Fig. 5.

Changes in cardiac Na^+ current in ACE 8/8 cardiac myocytes. **A:** current-voltage curves for cardiac myocytes derived from WT and ACE 8/8 mice. Values are means $\pm$ SE ($n = 3$ animals and 10 cells for each group). There are no statistically significant differences in slope conductance. V_m , membrane potential. **B:** activation kinetics in ACE 8/8 and WT myocytes. Data are fitted to the Boltzmann activation curve: $I/I_{\max} = 1/[1 + e^{(V_h - V)/k}]$, where V_h is the gating potential, k is the slope, and $I/I_{\max}$ is the ratio of the current at each holding potential to the maximum current calculated using a linear fit of the reversal region. Activation curve for ACE 8/8 mice is shifted to the left ($V_h = -37$ mV, $n = 10$) compared with WT cells ($V_h = -32$ mV, $n = 10$, $P < 0.001$). **C:** alterations in inactivation kinetics in ACE 8/8 compared with WT myocytes. Data are fitted to the Boltzmann inactivation curve described in **B**. Similar to activation kinetics, inactivation curve for ACE 8/8 cardiac myocytes is shifted to the left ($V_h = -65$ mV in ACE 8/8 myocytes, $n = 11$) compared with WT cells ($V_h = -55$ mV, $n = 13$, $P < 0.001$).

Fig. 6.

Alterations in connexin mRNA abundances with cardiac ACE expression. Relative atrial and ventricular mRNA abundances for Cx40 (A), Cx43 (B), Cx45 (C), Kcnd2 (D), Cacna1 (E), and Scn5a (F) determined by quantitative real-time PCR are shown for WT ($n = 4$), HZ ($n = 4$), and ACE 8/8 ($n = 4$) mice. Relative gene expression was assayed and normalized to endogenous β -actin. * $P < 0.01$ vs. WT.

Fig. 7.

Effect of 1-heptanol perfusion on electrical activity. *A*: representative example of effect of perfusion with the gap junction blocker 1-heptanol on pseudo-ECG recording from an isolated arterially perfused WT mouse heart. Abscissa reflects time from the moment perfusion started. au, arbitrary units. *B*: 1 mM heptanol-induced reduction of QRS amplitude in WT mouse hearts was consistent and statistically significant ($n = 5$, $P = 0.001$). *C*: 1 mM heptanol does not affect other ECG parameters, such as basal cycle length (BCL), P-R interval (PR), and QRS width (QRSw).

Table 1

Results from intracardiac electrophysiology

	WT (<i>n</i> = 7)	HZ (<i>n</i> = 7)	ACE 8/8 (<i>n</i> = 6)
Waves, mV			
Atrial	0.27±0.06	0.17±0.04	0.03±0.01 [*]
Ventricular	0.84±0.21	0.78±0.19	0.20±0.15 [*]
Intervals, ms			
AV	42±1	40±3	118±13 [*]
AH	33±2	30±2	86±10 [*]
HV	9±1	10±1	35±11 [*]

Values are means ± SE. Atrial and ventricular waves are maximum amplitudes of intracardiac atrial and ventricular activity, respectively. WT, wild-type mice; HZ, heterozygous mice; ACE 8/8, homozygous mouse model of renin-angiotensin system activation that uses cardiac-restricted angiotensin-converting enzyme; AV, AH, and HV, atrioventricular, atrio-Hisian, and Hisian-ventricular, respectively. Beat with the shortest atrioventricular duration was chosen for calculations in ACE 8/8 mice.

^{*}
 $P < 0.01$ vs. WT.