Dynamic changes in conduction velocity and gap junction properties during development of pacing-induced heart failure


Division of Cardiology and Institute for Computational Medicine, Johns Hopkins University, Baltimore, Maryland

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Akar FG, Nass RD, Hahn S, Cingolani E, Shah M, Hesketh GG, DiSilvestre D, Tunin RS, Kass DA, Tomaselli GF. Dynamic changes in conduction velocity and gap junction properties during development of pacing-induced heart failure. Am J Physiol Heart Circ Physiol 293: H1223–H1230, 2007. First published April 13, 2007; doi:10.1152/ajpheart.00079.2007.—End-stage heart failure (HF) is characterized by changes in conduction velocity (CV) that predispose to arrhythmias. Here, we investigate the time course of conduction changes with respect to alterations in connexin 43 (Cx43) properties and mechanical function during the development of HF. We perform high-resolution optical mapping in arterially perfused myocardial preparations from dogs subjected to 0, 3, 7, 14, and 21 days of rapid pacing to produce variable degrees of remodeling. CV is compared with an index of mechanical function [left ventricular end-diastolic pressure (LVEDP)] and with dynamic changes in the expression, distribution, and phosphorylation of Cx43. In contrast to repolarization, CV was preserved during early stages of remodeling (3 and 7 days) and significantly reduced at later stages, which were associated with marked increases in LVEDP. Measurements of differentially phosphorylated Cx43 isoforms revealed early, sustained downregulation of pan-Cx43 that preceded changes in CV and LVEDP, a gradual rise in a dephosphorylated Cx43 isoform to over twofold baseline levels in end-stage HF, and a late abrupt increase in pan-Cx43, but not dephosphorylated Cx43, localization. These data demonstrate that 1) CV slowing occurs only at advanced stages of remodeling, 2) total reduction of pan-Cx43 is an early event that precedes mechanical dysfunction and CV slowing, 3) changes in Cx43 phosphorylation are more closely associated with the onset of HF, and 4) Cx43 lateralization is a late event that coincides with marked CV reduction. These data reveal a novel paradigm of remodeling based on the timing of conduction abnormalities relative to changes in Cx43 isoforms and mechanical dysfunction.

END-STAGE HEART FAILURE (HF) is characterized by major electrophysiological changes that render the myocardium susceptible to lethal ventricular arrhythmias (20). Among these are a prolongation of the QT interval and average action potential (AP) duration (APD) (10, 19), an increase in the spatial gradient of repolarization across the ventricular wall (2, 13), and a decrease in conduction velocity (CV) (3). The cellular mechanisms underlying conduction slowing in HF include decreased expression of the principal ventricular gap junction protein connexin 43 (Cx43); however, this alone does not fully account for the magnitude of CV slowing in the canine tachycardia pacing model of HF (3), suggesting a prominent role for additional factors.

Beardslee et al. (8) demonstrated that 30 min of global no-flow ischemia in the isolated perfused rat heart led to a reduction in gap junction coupling resulting from an overall dephosphorylation of Cx43. More recently, we (3) and others (1) demonstrated an increase in the expression of a hypo- or dephosphorylated fraction of Cx43 on a PKC consensus site in end-stage HF that could contribute to the impairment of gap junction function and reduction of CV.

Interestingly, despite knowledge of altered electrophysiological properties, including CV slowing in end-stage HF, little is known about the time course of conduction changes and their relationship to altered expression and distribution of differentially phosphorylated Cx43 isoforms during the progression to HF. Therefore, in the present study, we sought to determine the temporal relationship between dynamic changes in conduction and Cx43 properties during the time course of tachycardia-induced HF.

Using high-resolution optical AP mapping, we measured changes in CV in arterially perfused canine wedge preparations isolated from unpaced dogs and dogs subjected to 3, 7, 14, and 21 days of rapid pacing to produce variable degrees of ventricular remodeling and dysfunction. Using a combination of confocal microscopy and immunohistochemical staining, we then compared the time course of changes in conduction properties with underlying changes in the expression and localization of differentially phosphorylated Cx43 isoforms.

We demonstrate that changes in CV occur with a distinct time course during the development of tachycardia-induced HF compared with APD. In contrast to changes in APD, which occurred rapidly after the onset of pacing, conduction was delayed only at later stages of remodeling. Interestingly, early reduction in total Cx43 expression did not result in CV slowing. Finally, what appeared as a systematic replacement of Cx43 by a dephosphorylated isoform of the protein, specifically at the intercalated disk, correlated more closely with CV slowing at advanced stages of remodeling.

METHODS

Experimental Models

All procedures involving the handling of animals were approved by the Animal Care and Use Committee of the Johns Hopkins University and adhered to National Institutes of Health guidelines. Dogs were instrumented and chronically paced as previously described (3, 10). Briefly, after insertion of a transvenous pacemaker, adult male mon-
gerd dogs (n = 22) were paced from the right ventricular apex at 250 beats/min for 3, 7, 14, or 21 days. Dogs paced for 21 days uniformly exhibited signs of HF, such as anorexia, lethargy, ascites, tachypnea, and muscle wasting. Before euthanasia, left ventricular (LV) end-diastolic pressure (LVEDP) was hemodynamically measured using a Millar catheter. Dogs were subsequently killed, and their hearts were rapidly excised for tissue isolation. Arterially perfused anterior LV wedge preparations were isolated and successfully prepared from animals paced for 3 days (n = 3), 7 days (n = 5), 14 days (n = 4), and 21 days (n = 5) and from unpaced controls (i.e., 0 day, n = 5), as previously reported (2, 3, 5, 22, 23). Preparations were paced via a unipolar lead, and a volume-conducted electrocardiogram was recorded. Tissue sections adjacent to the canine wedges were isolated and prepared as previously described for immunofluorescence staining with commercially available antibodies that recognize all phosphorylated and nonphosphorylated isoforms of Cx43 (pan-Cx43) or a form that is dephosphorylated at Ser368 (a consensus PKC site) of Cx43 (dephosphorylated Cx43). We previously demonstrated that the endocardial layer exhibited the most profound changes in Cx43, as well as other calcium-handling [sarco(endo)plasmic reticulum Ca2+/ATPase (SERCA2a) and phospholamban] and stress-related (phosphorylated ERK) molecules, in this model of pacing-induced HF (18); thus we focused our protein chemistry and immunohistochemical analysis on this layer.

Optical AP Mapping

We designed a system capable of recording 464 optical APs with high spatial, temporal, and voltage resolutions from the arterially perfused canine LV wedge preparation during steady-state pacing with a unipolar silver needle electrode (10 mm long, 30 gauge, Grass-Telefactor) at a wide range (0.1–5.0 Hz) of pacing rates and programmed electrical stimulation (3).

Briefly, wedges of myocardium dissected from the LV free wall were arterially perfused with normal Tyrode solution at 36 ± 1°C. After they were stained with the voltage-sensitive dye di-4-ANEPPS (15 μmol/l for ~8 min), the wedges were stabilized against a flat imaging window in a custom-designed, temperature-controlled chamber (3). Emitted light from the stained wedges (excited by 515 ± 5 nm light) was long-pass filtered at 630 nm and focused onto a 464-element photodiode array with a custom-designed optical microscopy containing a high-numerical-aperture lens and a dichroic mirror, allowing delivery of the excitation light to and collection of emitted light from the preparation along the same optical path in an epifluorescence configuration and, thereby, maximizing signal-to-noise characteristics of recorded optical signals (3).

Electrophysiological Measurements

CV. For assessment of CV, APs were recorded during steady-state pacing at a basic cycle length of 1,000 ms, as previously described in detail (3, 9). Local activation time at each site was defined as the maximum first derivative during the upstroke of the AP at each site. To minimize artificial increases in CV resulting from simultaneous capture of tissue in the vicinity of the needle electrode, we set the stimulus strength to just above the pacing threshold (at 1-ms pulse duration). This was periodically verified throughout the experiment. Velocity vectors, measured as the magnitude and direction of local CV at each site, were derived from the activation time of each pixel relative to those of its neighbors, scaled by the distance to neighboring sites. We measured CV along the axis of impulse propagation by averaging the magnitude of the velocity vectors along that direction (3).

APD. Repolarization time was defined as the point of maximum second derivative during the repolarization phase, as previously described (2, 5). APD was defined as the difference between the repolarization and activation times at each site during baseline pacing at a frequency of 1 Hz.

Immunohistochemistry

Double immunolabeling was performed using FITC- and rhodamine red-X (RRX)-labeled secondary antibodies. To reduce variability between preparations, we immunolabeled all sections simultaneously using identical dilutions of primary and secondary antibodies. Tissue sections (1 cm²) were frozen in liquid nitrogen and embedded in Tissue-Tek OCT compound (Sakura FineTek, Torrance, CA). Cryosections (10 μm thick) were cut and melted on Fisher Superfrost Plus slides and stored at -80°C. On the day of the experiment, the slides were placed in a moist chamber and allowed to warm to room temperature for 30 min. Preparations were fixed with 4% paraformaldehyde (pH 7.4) for 15 min and then blocked with 1% BSA and 2% normal goat serum (Sigma) in PBS for 90 min. Slides were then washed with PBS and stained with the following primary antibodies for 3 h: anti-pan-Cx43 (MAB 3068, Chemicon, Temecula, CA; 1:250 dilution), anti-dephosphorylated Cx43 (Zymed; 1:250 dilution), and polyclonal rabbit anti-cadherin (catalog no. ZYPC7, Zymed; 1:500 dilution). Slides were then washed with PBS and stained with the following secondary antibodies: FITC-conjugated AffiniPure goat anti-mouse IgG (Jackson ImmunoResearch, West Grove, PA; 1:100 dilution, 495- and 525-nm wavelengths, 50 μl) and RRX-conjugated AffiniPure goat anti-rabbit IgG (Jackson ImmunoResearch; 1:100 dilution, 570- and 590-nm wavelengths, 50 μl) for 90 min. Slides were finally washed in PBS and mounted with 15 μl of Vectashield + 4’,6-diamidino-2-phenylindole (Vector Labs) and stored at 4°C. In a subset of experiments, slides were also stained with 50 μl of tetramethylrhodamine isothiocyanate-conjugated phalloidin antibody (Sigma; 1:500 dilution) for 30 min at room temperature, thereby serving as an intracellular marker that facilitated the identification of cell borders.

Confocal Microscopy

Images were collected on a confocal microscope (Axiovert 100, Zeiss). Parameters controlling image properties, including zoom, pinhole dimensions, and objective and laser power, were kept constant for image acquisitions, as previously described (12). The red emission of RRX was collected at 605 ± 25 nm, and the green emission of FITC was recorded at 525 ± 25 nm. The 512 × 512 pixel 8-bit gray-scale images of the two emission channels were collected simultaneously and stored as previously described (6). At least five distinct images from each specimen were acquired and analyzed.

Image Analysis

FITC and RRX channels were thresholded to match immunolabeling patterns, as previously described (3, 17). N-cadherin pixels overlapping Cx43 pixels were counted in an automated fashion. The areas of thresholded Cx43 densities were measured using the Image J software package. Pixel-by-pixel colocalization analysis was used to explore the degree of colocalization between N-cadherin and Cx43. The expression of total Cx43 and Cx43 dephosphorylated at Ser368 was also quantified in an automated fashion and reported as percent area normalized to the total cell surface area per field, as previously described (8).

Western Blotting

Tissue sections adjacent to the optically mapped wedges were dissected and frozen. Proteins were prepared as previously described (3, 4). All samples were run in duplicate or triplicate on 12% Tris·HCl precast gels (Bio-Rad, Hercules, CA) in 25 mM Tris, 192 mM glycine, and 0.1% (wt) SDS running buffer. A standard control sample was run on all gels to allow for comparisons across gels. Primary antibody incubations were performed overnight at 4°C as previously...
described (8) using a commercially available antibody to measure pan-Cx43 (Chemicon; 1:1,000 dilution). Secondary horseradish peroxidase-conjugated antibodies were obtained from Jackson ImmunoResearch. Membranes were exposed and developed using enhanced chemiluminescence (Amersham Pharmacia Biotech, Piscataway, NJ) according to the manufacturer’s instructions. Autoradiograms were scanned, and band densities were quantified using the ImageQuant software package (Molecular Dynamics, Sunnyvale, CA). Cx43 band densities were normalized to the density of GAPDH to correct for variations in protein loading. Band intensities were expressed quantitatively as arbitrary optical density units, which correspond to the laser densitometric Cx43 protein band intensity after background subtraction divided by the GAPDH signal intensity for the same sample.

**Statistical Analysis**

Measurements between each of the paced groups were first compared with unpaced controls using the Student’s t-test. One-way ANOVA followed by Bonferroni’s, Dunn’s, and Tukey’s multiple-comparisons tests were performed to compare all groups. A post test for linear trend of dephosphorylated Cx43 measurements using confocal microscopy was also performed with the Graphpad Prism software package. Summary data are presented as means ± SD. Differences were considered significant at *P* < 0.05.

**RESULTS**

**Time Course of Changes in Hemodynamic Properties**

We assessed the time course of changes in global LV function during the time course of pacing-induced HF by measuring the LVEDP of dogs in each group. LVEDP increased significantly (*P* < 0.05) after 7 days of pacing (Fig. 1). LV dysfunction, as previously defined in the canine tachycardia pacing model (14, 21), was established after 14 days of pacing, when LVEDP was increased more than threefold over baseline control values. There were no significant changes in LVEDP between the 14- and 21-day-paced animals (Fig. 1).

**Time Course of Changes in Conduction During Development of HF**

We then evaluated the time course of changes in AP conduction in wedge preparations isolated from unpaced dogs and dogs paced for 3, 7, 14, and 21 days. Depolarization isochrone maps from representative dogs in each group are shown in Fig. 2. Although CV was relatively rapid in unpaced dogs and dogs paced for 3 and 7 days, it was moderately (~17%) slowed in dogs paced for 14 days and markedly (~42%) slowed in dogs paced for 21 days, as evidenced by the crowding of isochrone lines (Fig. 2A), the prominent delays between AP upstrokes of adjacent sites compared with normal hearts (Fig. 2B), and quantitatively (Fig. 2C). Moreover, the sequential spread of membrane depolarization further demonstrates the relative preservation of CV in the dogs paced for 3 and 7 days compared with the moderate and pronounced slowing of CV in the dogs paced for 14 and 21 days, respectively (supplemental data for this article are available online at the American Journal of Physiology Heart and Circulatory Physiology website).

APD prolongation is a hallmark of electrical remodeling and HF, so we investigated the time course of changes in APD during rapid pacing. This provided a positive control that confirmed the efficacy of the pacing paradigm in producing electrophysiological effects characteristic of HF remodeling. In contrast to CV, APD changed very rapidly with time of pacing (Fig. 3), which demonstrates significant prolongation of APD after only 3 days of pacing. After only 7 days of pacing, APD was comparable to that in dogs paced for 21 days (HF; *P* = not significant).

**Time Course of Changes in Expression of Pan-Cx43**

To investigate the relationship between electrophysiological remodeling at the tissue level and underlying molecular mechanisms, we investigated the time course of changes in the expression of total Cx43 (pan-Cx43) in dogs from all groups. Representative Western blots of pan-Cx43 and GAPDH and an aliquot of each lane were exposed to film. Quantitative analysis of band intensities was performed with the ImageQuant software package. Summary data are presented as means ± SD. Differences were considered significant at *P* < 0.05 (Fig. 4). Although total Cx43 expression was decreased (~40%, *P* < 0.05) after only 3 days of rapid pacing, it was not further significantly (7, 14, and 21 days vs. 3 days) reduced with longer pacing (Fig. 4). A qualitatively similar pattern of early (i.e., 3 days) reduction in Cx43 was also observed in epicardial and midmyocardial tissue sections (not shown).

**Time Course of Changes in Distribution of Pan-Cx43**

In addition to measuring expression of Cx43, we also investigated its distribution in endocardial tissue sections isolated from unpaced dogs and dogs paced for 3, 7, 14, and 21 days. The relative colocalization of total Cx43 with the intercalated disk protein N-cadherin was ~65% in normal hearts as determined using quantitative image analysis of confocal microscopic sections (Fig. 5A). Interestingly, the degree of colocalization between total Cx43 and N-cadherin was not significantly altered in dogs paced for 3, 7, or 14 days (Fig. 5A). In contrast, tissue sections from animals paced for 21 days exhibited a significant (22%, *P* = 0.0168) reduction in the extent of colocalization between pan-Cx43 and N-cadherin (Fig. 5A). A similar reduction of total Cx43 colocalization with N-cadherin was also observed in epicardial tissue sections (*P* = 0.0187, 21 days vs. unpaced) and when all transmural layers were combined (*P* = 0.0052, 21 days vs. unpaced). In addition, we also quantified the distribution of pan-Cx43 in all groups of animals by measuring the degree of lateralization of pan-Cx43 in tissue sections...
that were costained with the intracellular marker phalloidin, which allowed the identification of cell borders. Similarly, the percentage of pan-Cx43 outside the end-to-end junction between myocytes (i.e., lateralized) was unchanged, except in the 21-day-paced dogs, which exhibited a marked (63%, \( P < 0.001 \)) increase (Fig. 5B). Representative images of the distribution of N-cadherin (top, red) and pan-Cx43 (bottom, green) in endocardial tissue sections (Fig. 5C) illustrate extensive overlap.

Fig. 2. A: representative depolarization isochrone maps of wedge preparations from unpaced dogs and dogs paced for 3, 7, 14, and 21 days. B: superimposed action potential (AP) upstrokes from equidistant sites along the main axis of impulse propagation in a representative preparation from each group. C: average conduction velocity (CV) in all groups. *\( P < 0.05 \) vs. 0 day.

Fig. 3. Representative optical APs recorded from each group. Average endocardial AP duration (APD) was measured in preparations from unpaced dogs and dogs paced for 3, 7, 14, and 21 days. BCL, basic cycle length. *\( P < 0.05 \) vs. 0 day.

Fig. 4. Expression of pan-connexin 43 (Cx43) in endocardium of unpaced dogs and dogs paced for 3, 7, 14, and 21 days. Top: representative Western blot of pan-Cx43 and GAPDH. Bottom: average data showing an early and sustained decrease in expression of pan-Cx43 after only 3 days of pacing. *\( P < 0.05 \) vs. 0 day.
in the location of pan-Cx43 and N-cadherin signals in preparations from unpaced dogs and dogs paced for 14 days, but not in dogs paced for 21 days (white circles).

**Expression and Distribution of Differentially Phosphorylated Cx43 Isoforms**

We previously reported that end-stage HF produced a dramatic change in the phosphorylation state of Cx43 (3). Therefore, we measured the time course of changes in the expression of dephosphorylated (at Ser368) immunoreactive Cx43 signal in tissue sections from unpaced animals and animals paced for 3, 7, 14, and 21 days to determine the temporal relationship of changes in dephosphorylated Cx43 to changes in mechanical function and CV. In sharp contrast to pan-Cx43, dephosphorylated Cx43 expression increased with time of pacing in endocardial (Fig. 6) and epicardial (not shown) tissue sections. A marked (>2-fold) increase ($P < 0.05$) in dephosphorylated Cx43 expression at 21 days of pacing coincided with the dramatic decrease in CV at that stage.

Quantitative analysis of the normalized immunoreactive signal outside the end-to-end junctions relative to the total immunoreactive signal (an index of protein lateralization) in phalloidin-stained sections (Fig. 7A) revealed a significant ($P = 0.0002$) increase in pan-Cx43 lateralization after 21 days of pacing compared with unpaced control dogs (Fig. 7B, left), consistent with the decrease in percent colocalization between pan-Cx43 and N-cadherin after 21 days of pacing (Fig. 5). In sharp contrast, there was no change in dephosphorylated Cx43 ($P = 0.29$, not significant) lateralization in dogs paced for 21 days compared with unpaced dogs (Fig. 7B, right).

**DISCUSSION**

HF is the leading cause of morbidity and mortality in the industrialized world, with an estimated 5,000,000 patients suffering from LV dysfunction in the United States alone (16). Ventricular remodeling associated with HF predisposes to arrhythmias and sudden cardiac death, even before significant clinical symptoms of LV dysfunction become apparent (16). Therefore, understanding the sequence of molecular events that occur during the early development of HF and lead to dynamic changes in the electrophysiological substrate may hold the key for developing novel antiarrhythmic strategies.

We previously showed that conduction slowing is a prominent feature of HF that contributes to the arrhythmogenic substrate (3). Interestingly, reduced expression of Cx43 alone does not fully explain slowing of CV in HF, arguing for the existence of additional factor(s) that may contribute to the reduced CV. In the present study, we related quantitative changes in CV during the time course of tachycardia-induced HF with dynamic changes in the level of expression and distribution of differentially phosphorylated forms of Cx43.
The main findings of the present report are as follows. 1) In contrast to APD prolongation, CV slowing occurs only after manifest LV dysfunction (Figs. 1–3). 2) Total reduction of Cx43 (pan-Cx43) occurs very early after the onset of pacing (Fig. 4), preceding changes in LVEDP and CV. 3) The distribution of Cx43 is preserved until the very late stages of remodeling (Figs. 5 and 7). 4) In “end-stage” HF, there is a dynamic shift in the phosphorylation state of Cx43 at the intercalated disk, where progressively more dephosphorylated Cx43 is expressed (Fig. 7).

Expression of Differentially Phosphorylated Cx43 Isoforms

The main goal of the present study was to track changes in CV during the time course of tachycardia-induced LV dysfunction and relate them to dynamic changes in Cx43 properties. Using Western blot, we previously demonstrated that immunoreactive Cx43 expression is decreased by ~30% in the LV of failing canine hearts (3). However, these changes alone do not explain the magnitude of CV slowing in this model of nonischemic cardiomyopathy (3, 17). We have demonstrated that the reduction in total Cx43 expression in this model occurs very early (after only 3 days of pacing) and, hence, precedes HF and CV slowing. Moreover, our findings regarding the increase in dephosphorylated Cx43 during the development of HF are consistent with earlier findings of enhanced dephosphorylated Cx43 protein content in end-stage HF by Western blot analysis (1, 3). Interestingly, the increase in dephosphorylated Cx43 appears to be gradual and, thus, might contribute to compromised cellular coupling and predispose to arrhythmias before the onset of symptomatic HF. Hence, regulation of Cx43 phosphorylation may represent a viable therapeutic antiarrhythmic target, which requires direct investigation.

A major finding of our study is that differentially phosphorylated isoforms of Cx43 undergo selective subcellular localization in HF. Although, in normal hearts, Cx43 is primarily located at the end-to-end junctions between myocytes (the intercalated disk), this localization is significantly altered in HF, inasmuch as pan-Cx43 is readily distributed at lateral cell membranes or the intracellular space (Fig. 5). Our data demonstrate that dephosphorylated Cx43, which is not abundant in normal (unpaced) hearts (Fig. 6), systematically replaces the phosphorylated protein at end-to-end junctions between myocytes as HF develops. Since dephosphorylation of Cx43 at PKC consensus sites is known to impair gap junction conductance, our results suggest that reduced coupling, specifically at the intercalated disk, is due at least in part to a change in gap junction function and not simply a reduction in Cx43 protein expression at that critical location. These results are consistent with previous data demonstrating reduced gap junction function, independent of reduced Cx43 expression in the epicardial border zone of healing myocardial infarcts (24).

Our data also indicate that the lateralization process observed in HF requires or causes the protein to be phosphorylated in situ at least at Ser368. As such, Cx43 phosphorylation may play a role in protein trafficking into and out of the intercalated disk region, consistent with an earlier report demonstrating hyperphosphorylation of Cx43 when it undergoes degradation and endocytosis after cell isolation (7). However, despite being phosphorylated, it appears that the lateralized Cx43 is non- or hypofunctional, since CV is uniformly reduced in the fast and slow directions of impulse propagation (i.e., along and across cardiac fibers) (3). Taken
together, our data point to the importance of changes in the spatial distribution and posttranslational modification of Cx43, in addition to alterations in protein abundance, in the genesis of conduction abnormalities and arrhythmias.

Clinical Perspective: Novel Paradigm of Ventricular Electrical Remodeling

Although recent studies have highlighted the importance of electrophysiological remodeling, including major changes in conduction (3) and repolarization (2, 10) properties in the genesis of arrhythmias in end-stage HF, little is known about the dynamic nature of these changes during the time course of rapid pacing leading to HF. To our knowledge, this is the first study to systematically relate the time course of ventricular conduction slowing to dynamic changes in the expression, localization, and phosphorylation of Cx43, which mediates the electrical coupling within the multicellular network during the development of HF.

Our data suggest a novel paradigm regarding ventricular electrophysiological remodeling during the time course of rapid-pacing-induced HF. Surprisingly, repolarization abnormalities (prolonged APD) develop relatively early in the remodeling process, preceding significant changes in LVEDP. In sharp contrast, CV slowing is a late event that occurs only with clear LV mechanical dysfunction. As such, these findings highlight the importance of conduction slowing as a signature of the late remodeled ventricle and suggest that early changes in APD may contribute to the progression of adverse remodeling and, thus, may serve as targets for early intervention.

Limitations

This canine model of rapid-pacing-induced HF is associated with electrical remodeling due to chronic rapid pacing as well as mechanical dysfunction. As such, our present study does not discriminate between the individual contributions of each to electrical remodeling. Also, although this model does not mimic the disease etiology of most HF (ischemic and nonischemic) patients, it provides a highly reproducible and robust experimental model that exhibits key hemodynamic, electrophysiological, and molecular changes associated with human HF.

Myocardial conduction is dependent on several important factors that are not related to gap junctions and that were not specifically investigated here. These include potential changes in the extracellular matrix (such as fibrosis) (11) and in the fast inward sodium current (15). Although these two factors may be critical determinants of CV slowing and conduction block in some forms of human HF and in other animal models of HF, their contribution is probably minor in this model, since we observed no evidence for a significant contribution of either factor to CV slowing after 3–4 wk of rapid pacing in previous studies (3, 10).

Finally, despite elucidation of the time course of individual changes in Cx43 properties relative to CV slowing, one cannot assume causality between any one of the individual changes and CV slowing. Instead, it is likely that a combination of the changes in the properties that we have described can give rise to the phenotypic changes in conduction that we report at each stage.
REFERENCES


