Journal of the American College of Cardiology
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Published by Elsevier Science Inc.

Vol. 39, No. 1, 2002
ISSN 0735-1097/02/$22.00
PII S0735-1097(01)01710-7

Relative Expression of Immunolocalized Connexins 40 and 43 Correlates With Human Atrial Conduction Properties

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OBJECTIVES The aim of this study was to determine the relationship between immunolocalized gap-junctional proteins and human atrial conduction.

BACKGROUND As a determinant of intercellular conductance, gap-junctional coupling is considered to influence myocardial conduction velocity. This study tested the hypothesis that the quantity of immunodetectable atrial gap-junctional proteins, connexin40 (Cx40) and connexin43 (Cx43), are related to atrial conduction velocity in humans.

METHODS Epicardial mapping was performed on 16 patients undergoing cardiac surgery using an array of 56 unipolar electrodes. The conduction velocity was measured over the right atrial free wall during sinus rhythm and at a paced cycle length 500 ms. A biopsy from this region was excised for quantitative confocal immunodetection of Cx40 and Cx43.

RESULTS There was no correlation between conduction velocity and Cx43 signal or total connexin signal (Cx40 + Cx43). Connexin40 signal was inversely correlated with conduction velocity ($p = 0.036$). However, the relative quantity of connexin immunolabeling (expressed as Cx40/[Cx40+Cx43] or the inverse equivalent Cx43/[Cx40+Cx43]) was strongly associated with conduction velocity during sinus rhythm, such that, as the proportion of Cx40 signal increased (and that for Cx43 decreased), the conduction velocity decreased ($p < 0.005$, $r = -0.66$). Furthermore, with paced atrial activation at 500 ms cycle length, the relative quantity of connexin labeling (Cx40/[Cx40+Cx43]) correlated with the rate-related change in atrial conduction velocity ($p < 0.02$, $r = 0.59$).

CONCLUSIONS In human right atrium, conduction velocity is inversely related to immunodetectable Cx40 levels. The relative level of connexins 40 and 43 signal is strongly associated with atrial conduction properties, suggesting that interactions between the two connexins may result in novel coupling properties. (J Am Coll Cardiol 2002;39:116–23) © 2002 by the American College of Cardiology

The gap junction is the cell membrane specialization responsible for low resistance intercellular coupling and is one of the determinants of myocardial conduction velocity (1,2). Gap junctions contain multiple channels, each consisting of two hemichannels, termed connexons, each constructed from six connexin proteins. Connexins are a multigene family of proteins, which have a high degree of molecular homology but form gap-junctional channels with different functional properties (3–6). In the human atrium, the predominant connexins are connexin43 (Cx43) and connexin40 (Cx40) (7). Connexin45 has also been shown to be present at low levels (7). Gap junctions present rate-determining resistive discontinuities to propagation, and the functional properties conferred by the relative levels of the connexins expressed, therefore, are thought to influence the conduction properties of myocardium, and an alteration in connexin expression may predispose to arrhythmias (2–5,8,9).

At present, gene knockout mice are the principal in vivo model used to study the role of connexins in myocardial conduction. In these studies, the homozygous Cx40 knockout mouse has reduced atrial conduction velocity, but the heterozygote has normal conduction velocity (10,11). In Cx43 knockout mice, reduction in Cx43 expression slows ventricular conduction but does not appear to change atrial conduction velocity (12,13). These studies demonstrate that not only is connexin expression a determinant of conduction velocity but also that it is difficult to predict the effects of naturally occurring variation in connexin expression on human myocardial conduction.

Previous studies have shown that, in individual gap-junctional plaques, Cx40 and Cx43 may be coexpressed and interact in a complex manner with poorly understood implications for gap-junctional coupling (4,14). Therefore, with existing models it is difficult to predict the association between naturally occurring connexin expression and myocardial conduction properties.

By investigating the hypothesis that the quantity of the major atrial connexins, Cx40 and Cx43, as detected by immunoconfocal microscopy, correlates with conduction velocity in the human atrium, we aim to provide further insight into the role of gap-junctional remodeling in myocardial arrhythmogenesis.

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Manuscript received April 13, 2001; revised manuscript received September 10, 2001, accepted September 20, 2001.

Abbreviations and Acronyms

BSA = bovine serum albumin
Cx40 = connexin40
Cx43 = connexin43
FITC = fluorescein isothiocyanate
PBS = phosphate-buffered saline
TRITC = tetramethyl rhodamine isothiocyanate

METHODS

Study protocol. The study was performed on 16 patients undergoing coronary artery bypass grafting. Patients on antiarrhythmic drugs were excluded. Written informed consent was obtained from the patients, and the local ethics committee granted approval for the study. Standard anesthesia was followed by a midline sternotomy. Epicardial mapping was performed on the intact right atrial free wall before cardiopulmonary bypass. An array of 112 electrodes (3.5-mm interelectrode spacing, 16 × 7 electrodes) was positioned over the right atrial free wall guided by the sulcus terminalis ensuring that 56 electrodes covered the trabeculated free wall. An indifferent electrode was placed behind the left ventricle. A CardioMapp system (1-kHz sampling rate) was used to acquire data during sinus rhythm and pacing at 5 mA at a 500-ms interval from a site that closely simulated the sinus rhythm activation sequence. An excision biopsy (approximately 50 mm^3) was taken from the mapped region of the trabeculated atrial free wall.

Conduction velocity measurement. The activation time of each electrode and those of the immediate neighboring electrodes were used to calculate the conduction velocity by the method of triangulation (Fig. 1). The mean conduction velocity of all the triangles of neighboring electrodes over the trabeculated free wall was calculated for each beat. The mean of the mean conduction velocities for the three sinus and three paced beats was calculated.

Processing of specimens. The atrial biopsies were embedded in wax according to standard histologic procedures (15,16). Sections (10 μm) were immunolabeled after incubation in 0.1% trypsin solution. Labeling for each of the two connexins was performed on separate sections rather than double labeling to avoid potential steric hindrance of one antibody by the other.

Immunolabeling of Cx43. Sections were blocked with 0.1M L-lysine in phosphate-buffered saline (PBS) containing 0.1% Triton X-100 and then incubated in an optimized dilution of polyclonal rabbit antibody (E12H, termed HJ in earlier reports) in blocking agent (16). After washing, the sections were treated with a secondary anti-rabbit antibody tagged with CY3 fluorescent marker in blocking agent and mounted.

Immunolabeling of Cx40. The Cx40 antibody used was that developed against residues 255 to 270 of rat Cx40 (4,17). The antisera from three rabbits were affinity-purified and characterized by Western blotting and immunofluorescence of HeLa transfectants, expressing a range of connexins as previously described (17). The purified antisera gave a positive signal in Cx40-transfectants, and affinity purified S15C (R85) was used in this study as it was found to be optimal for wax-embedded human atrial tissue. Study sections were blocked with 1% bovine serum albumin (BSA) in PBS and incubated in S15C (R85), optimally diluted in blocking agent, and, after washing, the sections were treated with anti-rabbit CY3 in blocking agent and mounted.

Quantitative analysis. Immunolabeled sections were examined by confocal three-channel laser scanning microscopy using a Leica TCS 4D equipped with an argon/krypton laser and fitted with appropriate filter blocks for the detection of fluorescein isothiocyanate (FITC), tetramethyl rhodamine isothiocyanate (TRITC) and CY5 fluorescence. Six randomly selected fields were acquired for each section at ×40 magnification (field area = 60,140 μm^2) of a single optical slice (<1 μm). The TRITC filter detects the appropriate wavelength for CY3 fluorescence and, therefore, connexin labeling, but it also detects the nonspecific autofluorescence emitted at a range of wavelengths by connective tissue and lipofuscin. The FITC filter shows only the connective tissue and lipofuscin autofluorescence,

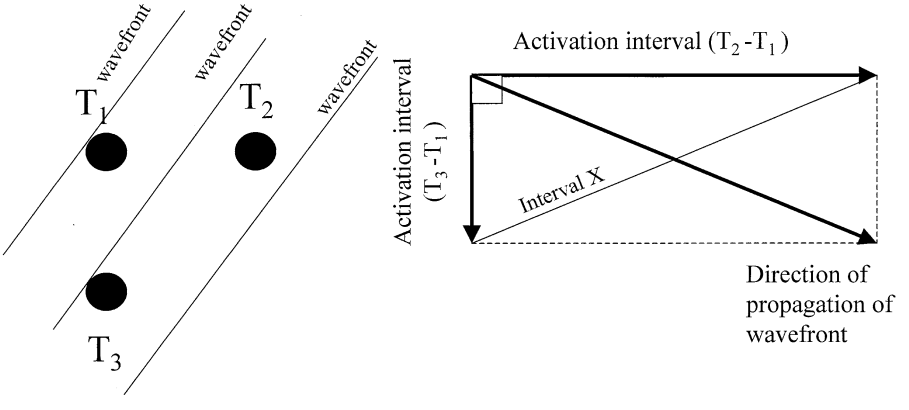

Figure 1. Method for calculating conduction velocity. Diagram shows three electrodes and their associated activation times (T_1 to T_3). Interval X represents the time taken for the activation wavefront to travel the interelectrode distance in the direction of propagation of the wavefront (geometric proof not shown). Therefore, the conduction velocity in the direction of propagation is the interelectrode distance divided by interval X.

Table 1. Intraoperative Right Atrial Conduction Velocities

Patient	Sinus Cycle Length (ms)	Mean of Mean Conduction Velocities in Sinus Rhythm (cm/s)	Mean of Mean Conduction Velocities During Pacing (cm/s)	Change in Velocity on Pacing (cm/s)
1	1,070	92.52 ± 2.03	79.92 ± 2.4	−12.6
2	1,274	64.31 ± 3.15	82.82 ± 5.37	18.51
3	1,144	95.71 ± 6.11	45.38 ± 4.38	−50.32
4	817	69.42 ± 0.54	81.56 ± 2.93	12.14
5	1,242	80.04 ± 5.21	79.69 ± 1.41	−0.34
6	645	115.36 ± 20.97	87.99 ± 0.6	−27.37
7	759	65.6 ± 0.83	59.78 ± 3.85	−5.81
8	1,103	86 ± 4.73	96.96 ± 5.53	10.96
9	858	60.82 ± 3.34	64.08 ± 8.92	3.26
10	800	95.41 ± 1.53	80.86 ± 5.5	−14.55
11	563	111.28 ± 3.65	97.62 ± 8.27	−13.65
12	890	68.92 ± 0.68	96.2 ± 5.45	27.28
13	1,340	80.38 ± 4.29	92.32 ± 9.08	11.93
14	1,111	78.18 ± 6.47	71.88 ± 8.4	−6.29
15	1,111	84.39 ± 2.58	54.33 ± 2.56	−30.05
16	1,372	75.29 ± 0.92	52.69 ± 2.45	−22.6
Total	1,006 ± 24	82.7 ± 16.1	76.5 ± 16.7	−6.2 ± 20.3

and the CY5 filter shows only lipofuscin autofluorescence. Using the relative signal intensity in three channels, it was possible on the composite image to separate and quantify connexin labeling using PC Image software. Immunohistochemical quantification of gap junctions, using the E12H (HJ) antibody, has been previously validated against electron microscopical quantification (16). The myocytic area was calculated by taking the difference between the myocardial field size and connective tissue autofluorescence. Connexin signal quantity was expressed per unit area of myocytic tissue. The role of endomysial connective tissue (between individual myocytes) and total connective tissue (endomysial and perimysial between-muscle bundles) in determining conduction velocity was also investigated (18). This was done by selecting six random fields and quantifying the total autofluorescence of connective tissue including both the endomysial and perimysial connective tissue per unit area of myocardial field. Then, on each of these randomly selected fields, a subselected field containing only endomysial connective tissue was quantified and represented per unit area of myocardial field.

Atrial samples from explanted transplant hearts. In the patients undergoing coronary artery surgery, it was only possible to take a single biopsy from the mapped region at the atriotomy site. In order to confirm the uniformity of pattern and quantity of connexin expression, three samples from sites at least 1cm apart were taken from the trabeculated right atrium of two explanted hearts. Immunolabeled images were acquired, as described for study patients, of randomly selected fields of both Cx40 and Cx43 labeled sections at ×16 (field area approximately 150,000 μm^2) and ×40 (field area approximately 60,000 μm^2) magnification to assess the pattern of connexin expression.

Immunogold electron microscopy. Samples were fixed with 2% formaldehyde and embedded in Lowicryl K4 M as previously described (14). Ultrathin sections were blocked with 1% BSA, followed by 1% gelatin then 0.02 M glycine in PBS and incubated with anti-Cx40 (S15C [R85]) in 0.5% BSA in PBS and then with anti-Cx43 (mouse monoclonal [Chemicon International, Essex, UK]) in PBS containing 0.5% BSA. This was followed by incubation with 10-nm-diameter gold/goat anti-rabbit and 5-nm-diameter gold/goat anti-mouse secondary antibodies (British Biocell International, Cardiff, UK) diluted in PBS. The sections were counterstained and examined with a Philips EM301 electron microscope.

Data analysis and statistical methods. Results are expressed as mean ± SD. Immunodetectable Cx40 and Cx43 were plotted against conduction velocity. To assess whether the relative signal of the two coexpressed connexins is related to overall conduction properties, the fractional content (Cx40/[Cx40+Cx43]) was plotted against conduction velocity. Correlation was analyzed by Pearson correlation test and considered significant if $p < 0.05$. If correlation was significant, a Pearson r value was also quoted.

RESULTS

Intraoperative mapping. Sixteen patients (mean age 62.4 ± 9.9 years, range: 43 to 76 years) were studied. The mean conduction velocity, measured over the trabeculated part of the right atrial free wall during sinus rhythm was 82.7 ± 16.1 cm/s and during pacing at 500 ms intervals was 76.5 ± 16.7 cm/s (Table 1). There was no correlation between intraoperative sinus cycle length and sinus conduction velocity. During pacing, 10 patients had a slowing of conduction velocity, and six had a small increase in conduction velocity. Overall, conduction velocity changed by −6.2 ± 20.3 cm/s.

Quantitative immunolabeling. Figure 2 illustrates Cx43 and Cx40 labeling in one of the patients. Using the quantitative immunolabeling method, mean Cx43 signal per

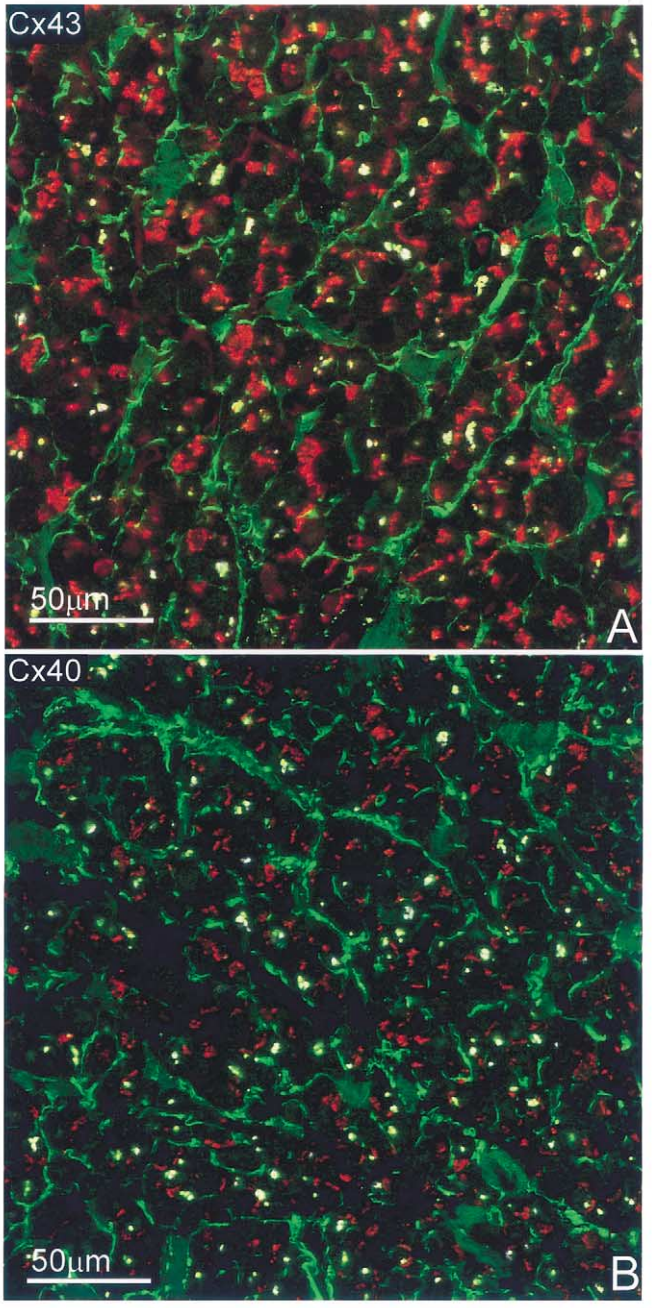

Figure 2. Three channel single optical slice images of Cx43 and Cx40 immunolabeling. This illustrates specific connexin labeling of Cx43 **(A)** and Cx40 **(B)**. "False" colors show specific connexin labeling **(red)**, connective tissue autofluorescence **(green)** and lipofuscin autofluorescence **(white)**. Connective tissue architecture is also apparent with both endomysial and perimysial strands.

unit area of myocyte was 0.045 ± 0.03 $\mu m^2/\mu m^2$, and mean Cx40 signal was 0.016 ± 0.010 $\mu m^2/\mu m^2$ (Table 2). As these values are dependent on the affinities of the antibodies used, they are not directly comparable.

Connexin40 labeling revealed distinct areas of low Cx40 signal, which were <10,000 μm^2 and, therefore, substantially smaller than the field area used for signal quantification (approximately 60,000 μm^2). Connexin43 labeling showed less variation and no detectable microscopic heterogeneity.

Table 2. Immunodetectable Connexin Amounts

Patient	Cx43 Signal ($\mu m^2/\mu m^2$)	Cx40 Signal ($\mu m^2/\mu m^2$)	Endomysial Connective Tissue ($\mu m^2/\mu m^2$)
1	0.0407 ± 0.0102	0.0208 ± 0.0075	0.0737 ± 0.0518
2	0.0339 ± 0.0044	0.0138 ± 0.0042	0.1238 ± 0.0539
3	0.0787 ± 0.0300	0.0162 ± 0.0080	0.0183 ± 0.0060
4	0.0402 ± 0.0066	0.0108 ± 0.0045	0.1371 ± 0.0570
5	0.0708 ± 0.0118	0.0155 ± 0.0041	0.1004 ± 0.0551
6	0.0796 ± 0.0055	0.0051 ± 0.0029	0.1113 ± 0.0792
7	0.0498 ± 0.0255	0.0204 ± 0.0043	0.0612 ± 0.0266
8	0.1306 ± 0.0090	0.0282 ± 0.0089	0.0879 ± 0.0236
9	0.0918 ± 0.0017	0.0430 ± 0.0133	0.0379 ± 0.0186
10	0.0089 ± 0.0045	0.0007 ± 0.0004	N/Q
11	0.0260 ± 0.0053	0.0050 ± 0.0007	N/Q
12	0.0314 ± 0.0003	0.0178 ± 0.0051	0.0419 ± 0.0169
13	0.0425 ± 0.0068	0.0238 ± 0.0063	0.0622 ± 0.0161
14	0.0478 ± 0.0033	0.0122 ± 0.0051	0.0355 ± 0.0179
15	0.0555 ± 0.0057	0.0109 ± 0.0046	0.0425 ± 0.0146
16	0.0329 ± 0.0133	0.0081 ± 0.0015	0.0911 ± 0.0579
Total	0.045 ± 0.03	0.016 ± 0.01	0.072 ± 0.03

Cx40 = connexin 40; Cx43 = connexin 43; N/Q = not quantifiable.

Figure 3 shows representative fields of Cx40 and Cx43 labeling from one of the explanted atria. Inspection of biopsies separated by at least 1cm from explanted hearts showed that these features of Cx40 and Cx43 labeling were consistent in different areas of the trabeculated right atrium of a given patient, and connective tissue content also appeared uniform throughout. Analysis of variance confirmed no significant difference in connexin signal between regions in each patient.

Assessment of colocalization. Double label immunogold electron microscopy (Fig. 4) demonstrated that Cx43 and Cx40 may be colocalized to individual gap-junctional plaques. A total of 22 gap-junctional plaques were studied, all of which contained label for both Cx40 and Cx43.

Immunodetectable connexin and conduction velocity. Although there was no apparent relationship between the quantity of Cx43 signal alone or of total connexin signal (Cx40+Cx43) and conduction velocity, an increase in the quantity of Cx40 signal was associated with a reduction in conduction velocity (Fig. 5A) during sinus rhythm ($p = 0.036$, $r = -0.52$). There was a strong correlation between the relative levels of connexin signal and conduction velocity during sinus rhythm, such that conduction velocity decreased as the proportion of Cx40 (expressed as Cx40/[Cx40+Cx43]) increased ($p < 0.005$, $r = -0.66$) (Fig. 5B) and, conversely, the proportion of Cx43 (Cx43/[Cx40+Cx43]) had a positive correlation with sinus conduction velocity ($p < 0.005$, $r = 0.66$) (Fig. 5C).

Conduction velocity during pacing at 500 ms intervals did not correlate with the quantity of Cx40, Cx43, total connexin signal or the relative immunodetectable signal of the two connexins. However, the relative immunodetectable signal (Cx40/[Cx40+Cx43]) correlated with the difference

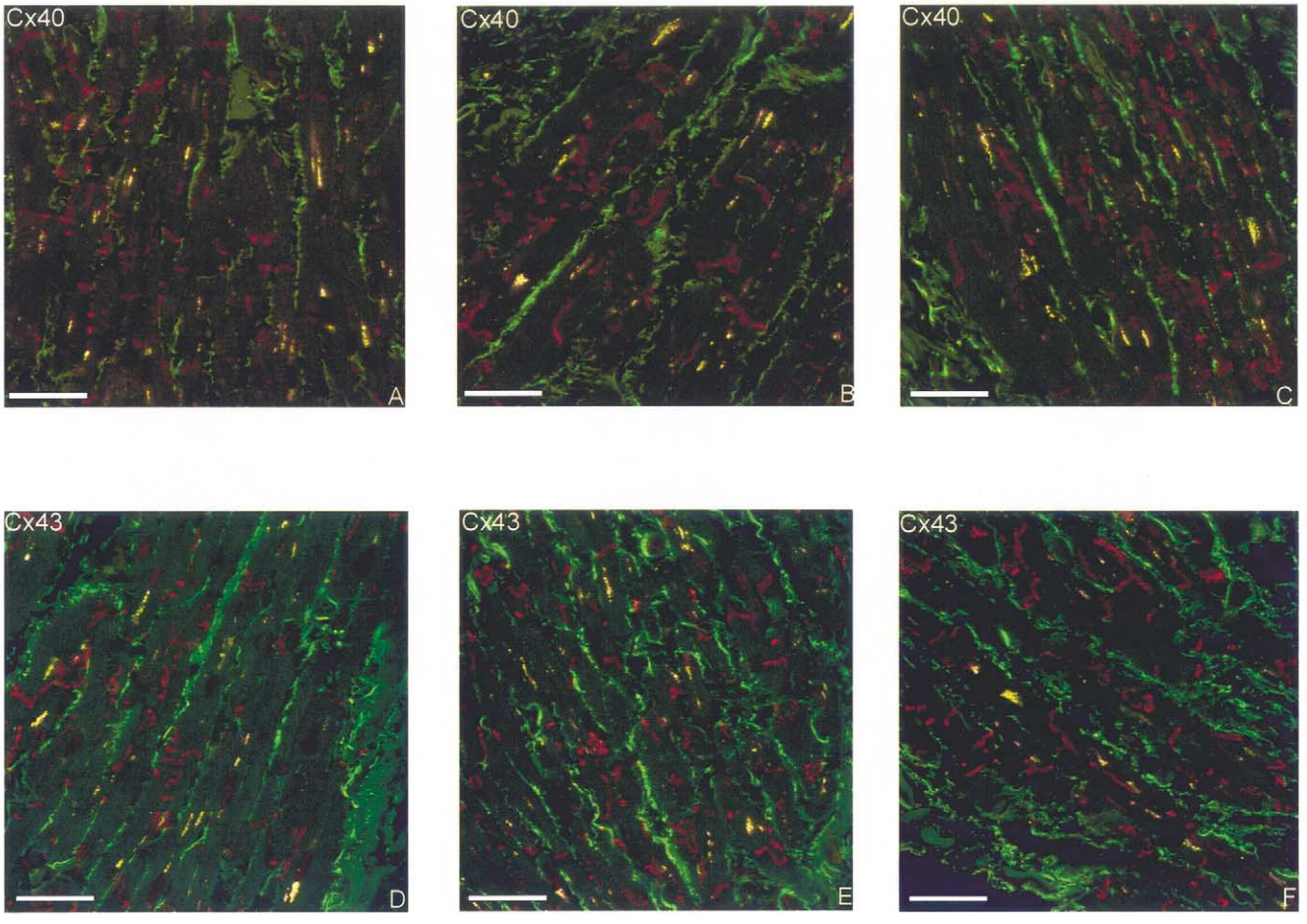

Figure 3. Three channel single optical slice images of sections from explanted atrial trabeculated myocardium. **(A to C)** are representative examples of Cx40 labeling and **(D to F)** are representative examples of Cx43 labeling from three atrial samples that were separated by at least 1 cm (bar = 50 μm).

in conduction velocity as measured during sinus rhythm and pacing (mean paced conduction velocity − mean sinus conduction velocity) such that a lower proportion of Cx40 was associated with a decrease in conduction velocity on pacing (Fig. 5D) ($p < 0.02$, $r = 0.59$). The quantities of Cx40, Cx43 and total connexin signals did not correlate with the changes seen during pacing.

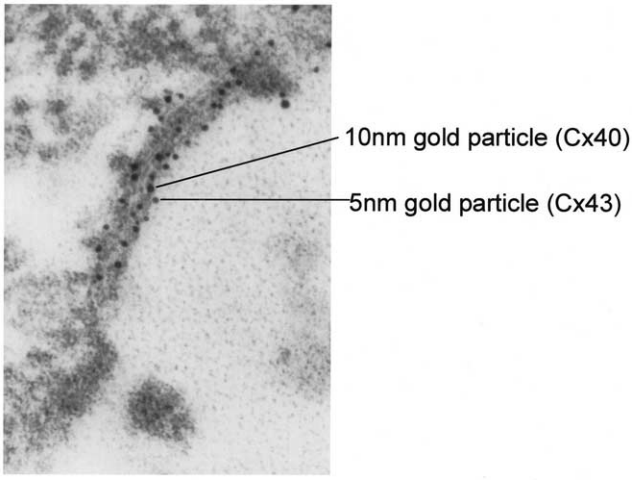

Figure 4. Transmission electron micrograph of a gap-junctional plaque in transverse section, with both 5 nm and 10 nm gold particles, indicating the presence of both connexins in this plaque.

Connective tissue. There was a marked variation in the quantity of connective tissue, but this did not correlate with the age of the patient. There was no correlation between either endomysial or total connective tissue and conduction velocity (Fig. 6). There was also no correlation between either endomysial or total connective tissue and connexin signal.

DISCUSSION

These results show that an increase in immunodetectable Cx40 signal (using the S15C [R85] antibody) and in Cx40 relative to total connexin signal (Cx40/[Cx40+Cx43]) is associated with a lower atrial conduction velocity during sinus rhythm. Although Cx43 signal alone did not correlate with conduction velocity, the relative Cx43 signal (Cx43/[Cx40+Cx43]), as would be expected, is associated with increasing sinus conduction velocity. The correlation between conduction velocity and the relative immunodetect-

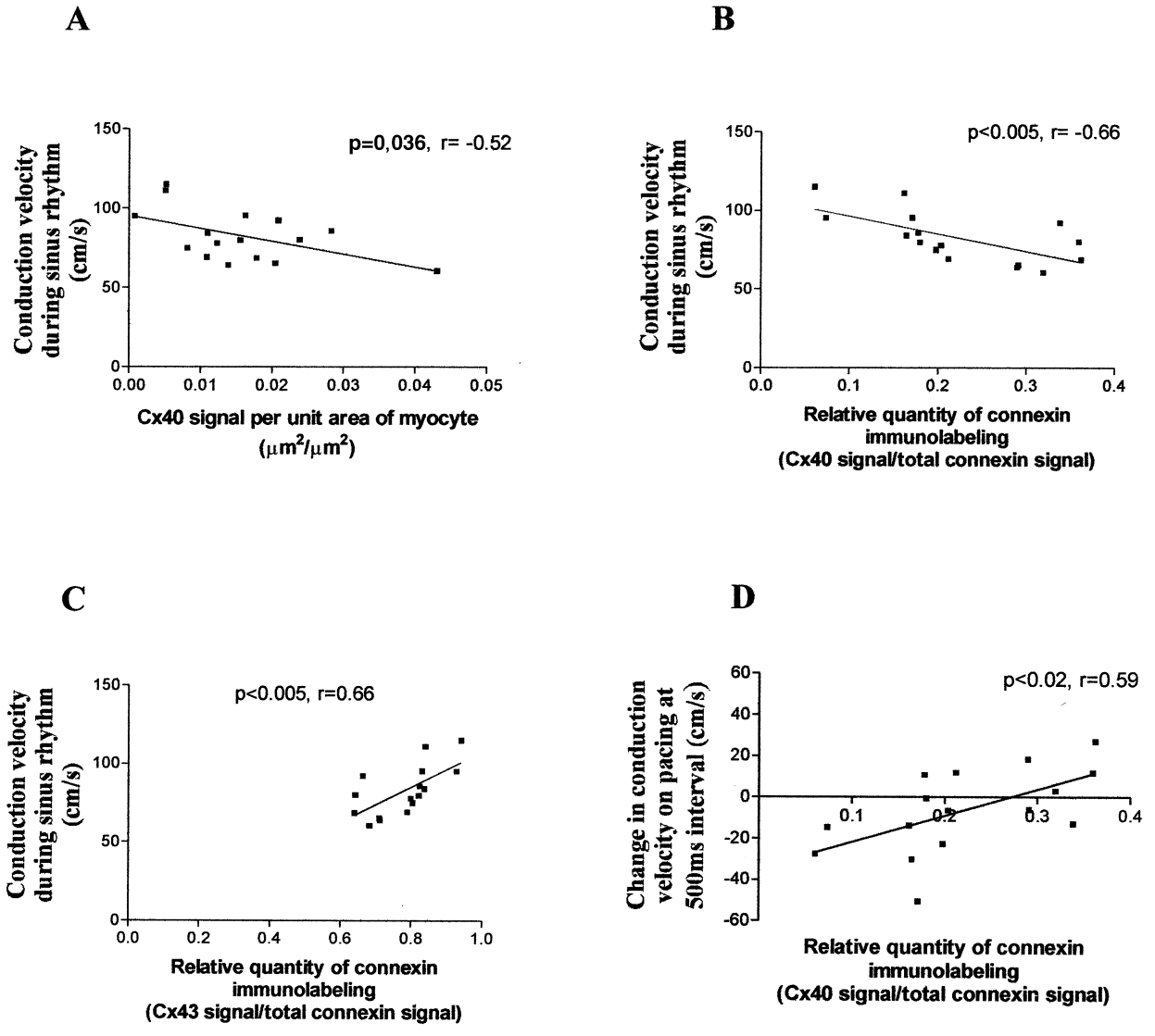

Figure 5. **(A)** Conduction velocity during sinus rhythm versus immunolocalized Cx40 expression. Conduction velocity during sinus rhythm versus relative quantity of immunolabeled Cx40 (Cx40/[Cx40+Cx43]) **(B)** and the inverse equivalent relative Cx43 signal (Cx43/[Cx40+Cx43]) **(C)**. **(D)** Change in conduction velocity on pacing versus relative quantity of immunolabeled connexins (Cx40/[Cx40+Cx43]).

able signal of the two connexins was stronger than that with Cx40 alone, suggesting that the relative expression may be the more important factor. Since it is well established that increased coupling is associated with increased velocity (1,19), in the intact human atrium with physiologic expression of connexins 40 and 43, it appears that an increased proportion of Cx40 (as detected by S15C [R85]) in the presence of Cx43 actually reduces coupling and, thus, conduction velocity.

Coexpression of connexins. The finding that a greater expression of immunodetectable Cx40 is associated with reduced conduction velocity may seem surprising, as in vitro studies have shown that channels composed entirely of Cx40 have a higher unitary conductance than those composed of Cx43 (20,21). However, when a pair of coupled cells expresses two connexin types, several types of channel with different combinations of connexins may, theoretically, be formed (homomeric homotypic, homomeric heterotypic, heteromeric homotypic or heteromeric heterotypic) (3,6). It is not established whether any of these occur in vivo in the human atria, but in vitro studies suggest the formation of functional gap-junctional channels constructed from both Cx40 and Cx43 (22–25). Cells coexpressing Cx40 and Cx43 have also been shown to be more susceptible to uncoupling than those cells expressing only one connexin type (25,26). Furthermore, in canine atrial cell pairs there is a range of gap-junctional unitary conductances, rather than discrete peaks, as would be expected from two populations of channels each of single connexin type (27). All these studies highlight the complex coupling effects of expressing more than one connexin, and interactions between connexins appear not to be straightforward. The results of this study in which conduction properties were measured in the intact human heart under as near physiological conditions as possible, therefore, could be explained by novel coupling

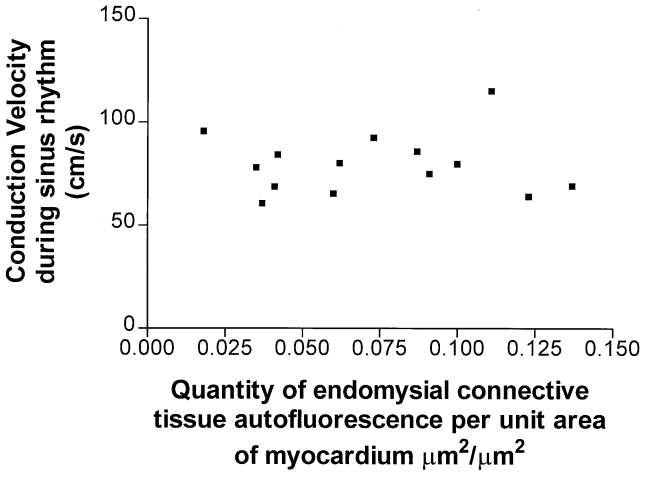

Figure 6. Endomysial connective tissue versus conduction velocity during sinus rhythm.

properties when Cx40 and Cx43 are coexpressed, such that an increasing proportion of Cx40 reduces coupling.

The method of connexin quantification used in this study measures the abundance of connexin that is accessible to a specific antibody and that is of sufficient focal concentration to be detectable at immunoconfocal microscopy. It is likely that the majority of quantified connexin label represents only that within intact gap-junctional membranes. By using intact tissue, this method of quantification may be affected by functionally relevant topological arrangement and steric interactions of the coexpressed connexins. By electron microscopy of double gold labeling, all gap-junctional plaques identified showed double labeling confirming extensive colocalization of Cx43 and Cx40 in human atria. Despite this, we cannot conclude that both connexins occur in single channels, only that it remains a possibility.

Connexin knockout mice. Previous studies relating connexin expression to conduction in the intact heart have been performed in Cx40 and Cx43 knockout mice. These studies showed that Cx43 reduction had no effect on atrial conduction velocity, that homozygous Cx40 knockout mice had reduced conduction velocity, but that atrial conduction in the heterozygous Cx40 knockout mice did not differ from wild type (10–12). In contrast, our data show that in humans, although immunodetectable Cx43 alone showed no direct correlation with conduction velocity, the relative quantity of connexin immunolabeling is related to the conduction properties of the trabeculated right atrium and, therefore, are suggestive of novel coupling properties when Cx40 and Cx43 are naturally coexpressed in the intact human heart. With increasing evidence that interactions between connexins are complex in cells coexpressing Cx40 and Cx43, there are limitations in attempting to extrapolate homozygous knockout mouse data to connexin coexpressing myocardium.

Conduction velocity and pacing. There are very limited data on the effects of coupling interval on human atrial conduction velocity (28). In this study, shortening the coupling interval to a uniform 500 ms did not result in a significant change in conduction velocity when analyzed as group data. However, in individual patients there were marked changes in conduction velocity from sinus rhythm to pacing, ranging from a decrease in conduction by 50 cm/s to an increase of 27cm/s. As a result, although connexin labeling was associated with conduction velocity during sinus rhythm, there was no such association with conduction velocity during pacing. However, the change in conduction velocity between sinus rhythm and pacing correlated with the relative quantity of immunolabeled connexin such that as the proportion of Cx40 decreases, the greater the degree of conduction slowing. Although changes in velocity were unlikely to be due to fiber orientation, as pacing was performed to simulate the pattern of activation in sinus rhythm, many other factors that can contribute to differences in epicardial conduction velocity, such as changes in resting potential, could not be measured under the experimental conditions used. However, this finding suggests that gap-junctional connexin expression is involved in the mechanism of adaptation of conduction velocity to rate, but further studies will need to be performed to elucidate the nature of this relation and potential mechanisms.

Connective tissue and conduction. Endomysial connective tissue (microfibrosis), which is present in the intercellular space, has been implicated in arrhythmogenesis due to local conduction disturbance (29). Our study did not demonstrate correlation between endomysial connective tissue content and conduction velocity, but the methods were not of sufficient resolution to differentiate between longitudinal and transverse propagation in myocardial fibers, and, therefore, a possible explanation for this is that propagation occurs primarily along the fiber axis whereby connective tissue septae would not be encountered by the wavefront.

Study limitations. It was not possible in this study to control for other factors that also contribute to the overall conduction velocity such as those that affect active membrane properties. Despite the expected scatter in the plots, correlations between the quantified connexin and conduction velocity were, nevertheless, evident. To investigate the possibility of interaction between the coexpressed connexins, the relative Cx40/(Cx40+Cx43) signal (and the inverse equivalent Cx43/[Cx40+Cx43]) was assessed. Unlike a simple ratio, this parameter will not magnify differences at extremes, but it remains subject to some of the problems of expressing relative data. By using polyclonal antibodies, the mean binding affinity of the Cx43 and Cx40 antibodies are unlikely to be very different. However, theoretical calculations showed that, even if the binding affinities differed by three orders of magnitude, the correlations persisted (data not shown).

The mapped area was larger than the biopsies that were studied, but the patterns of Cx40 and Cx43 labeling observed in mapped and explanted atria were similar, with an absence of significant macroscopic variation in the

pattern and quantity of connexin immunofluorescence between different regions of explanted trabeculated right atrium, suggesting that the pattern of labeling would also be similar throughout the mapped region.

We refer to immunodetectable connexins throughout the study and, as such, our findings are limited to the properties of the specific antibodies and the experimental conditions used. If heteromerization underlies our novel findings, the heteromerization may lead to altered binding properties of antibodies and, therefore, affect the quantity of label detected.

Conclusions. This study has shown that immunodetectable gap-junctional protein is related to conduction velocity in human myocardium. The finding that an increasing proportion of Cx40 signal is associated with a decreasing conduction velocity during sinus rhythm in the human atrium supports the concept that connexin interactions as well as individual connexin levels may contribute to modulation of electrical coupling in vivo. Additionally, the relation between the change in conduction velocity at shorter coupling intervals and the relative Cx40 and Cx43 signal may suggest a role for gap-junctional coupling in modulating the active cell responses to changing heart rate. These findings indicate a potentially complex relationship between connexins and action potential propagation.

Acknowledgments

The authors thank Mr. Ashok Cherian, Mr. David O'Regan, Mr. Brian Glenville and Mr. Rex Stanbridge who assisted with the atrial mapping and provided tissue specimens. The authors are also very grateful to Dr. Steven Coppen who developed and characterized the anti-Cx40 primary antibody.

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