Relationship Between Gap-Junctional Conductance and Conduction Velocity in Mammalian Myocardium

Paramdeep S. Dhillon, MRCP, PhD; Rosaire Gray, MRCP, PhD; Pipin Kojodjojo, MRCP, PhD; Rita Jabr, PhD; Rasheda Chowdhury, MSc, PhD; Christopher H. Fry, DSc, PhD; Nicholas S. Peters, FRCP, MD

Background—Gap junction resistivity, $R_j$, has been proposed as a key determinant of conduction velocity (CV). However, studies in connexin-gene knockout mice demonstrated significant CV slowing only with near-complete connexin deletion, and these findings led to the concept of a significant redundancy of myocardial gap junctions. We challenged this prevailing concept and addressed the hypothesis that there is a continuous relationship between $R_j$ and CV, each independently measured in human and guinea-pig myocardium.

Methods and Results—$R_j$ and CV were directly measured by oil-gap impedance and microelectrode techniques in human left ventricular myocardium from patients with hypertrophic cardiomyopathy and in guinea-pig atrial and ventricular myocardium before and during pharmacological uncoupling with 20-µmol/L carbenoxolone. There was a continuous relationship between $R_j$ and CV in human and guinea-pig myocardium, pre- and post-carbenoxolone ($r^2=0.946; P<0.01$). In guinea-pig left ventricle, left atrium, and right atrium, carbenoxolone increased $R_j$ by 28±9%, 26±16%, and 25±14% and slowed CV by 17±3%, 23±8%, and 11±4% respectively (all $P<0.05$ versus control). As a clinically accessible measure of local microscopic myocardial conduction slowing in vivo in the intact human heart, carbenoxolone prolonged electrogram duration in the right atrium (39.7±4.2 to 42.3±4.3 ms; $P=0.01$) and right ventricle (48.1±2.5 to 53.3±5.3 ms; $P<0.01$).

Conclusions—There is a continuous relationship between $R_j$ and CV that is consistent between cardiac chambers and across species, indicating that naturally occurring variations in cellular coupling can account for variations in CV, and that the concept that there is massive redundancy of coupling is not tenable. (Circ Arrhythm Electrophysiol. 2013;6:1208-1214.)

Key Words: carbenoxolone • electrophysiology • gap junctions

Mammalian myocardium is a functional syncytium of myocytes coupled by gap-junction (GJ) channels of connexin (Cx) proteins. Experiments with uniform 1- and 2-dimensional cultured strands, as well as computer simulations, show that these channels are important determinants of action potential (AP) propagation, concluding that increased GJ electric resistance ($R_j$) slows AP conduction velocity (CV).

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The situation in intact myocardial is less clear. One-dimensional cable theory predicts CV to vary inversely with the square root of total tissue axial resistance through the tissue, $R_c$, corroborated in mammalian ventricle by independent measurement of $R_j$ and CV. Axial resistance has 2 series components: from the sarcoplasm, $R_s$, and from GJs, $R_j$, each significantly contributing to $R_c$. Thus, $R_j$ would be expected to be an important and continuous determinant of CV in intact myocardium. At variance with this concept of a continuous relationship between $R_j$ and CV, studies in Cx43-gene knockout mice have shown CV slowing, measured by epicardial optical mapping, only when Cx43 deletion was virtually complete. This finding has been widely accepted as a general concept that there is massive redundancy of myocardial GJ coupling. In contrast, there are several reports of significant changes of CV associated with relatively small changes in Cx43 levels in diseased human myocardium and animal models of disease. However, the specific relationship between GJ resistance and CV has not been determined systematically, in large part, because the 2 variables have never been directly and independently measured in the same preparations, nor has the contribution from other potential contributory factors that may influence CV. Optical mapping does not allow a quantitative determination of the relationship between CV and $R_j$, as precise conduction pathways are unclear and $R_j$ cannot be measured. We therefore used techniques to measure directly CV and $R_j$ in a set of complimentary studies to test the hypothesis that in intact myocardium CV is a function of $R_j$ over a continuous range of values commensurate with moderate, naturally

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From the Imperial Centre for Translational and Experimental Medicine, Myocardial Function Section, Imperial College London and Imperial College NHS Trust, London, United Kingdom (P.S.D., P.K., R.C., N.S.P.); Department of Medicine, Whittington Hospital, London, United Kingdom (R.G.); and Faculty of Health and Medical Sciences, Institute of Biosciences and Medicine, University of Surrey, Guildford, United Kingdom (R.J., C.H.F.).

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Correspondence to Nicholas S. Peters, FRCP, MD, 4th floor Imperial Centre for Translational and Experimental Medicine, Hammersmith Campus, Du Cane Rd, London W12 0NN, United Kingdom. E-mail n.peters@imperial.ac.uk

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occurring ranges of coupling and uncoupling in pathological conditions. We used validated methods,3,14 to measure $R_e$ and CV and their general relationship in guinea-pig myocardium from different cardiac chambers with and without a GJ blocker, carbenoxolone, and in human ventricular myocardium excised from patients with hypertrophic cardiomyopathy. We also examined the effects of carbenoxolone on local, submillimeter propagation in intact human myocardial propagation as inferred from changes in bipolar electrogram duration during clinical electrophysiology studies to determine how the ex vivo findings are manifest in, and may therefore be inferred from, clinical measurements.

**Methods**

**Preparations: Guinea-Pig Myocardium**

Male Dunkin-Hartley guinea pigs (400–600 g) were euthanized and the hearts rapidly excised and immersed in preoxygenated Tyrode solution containing (mmol/L): NaCl 118, KCl 4.0, NaHCO3 24, NaH2PO4 0.4, MgCl2 1.0, CaCl2 1.8, glucose 6.1, Na pyruvate 5.0 (preagressed with 95% O2/5% CO2, pH 7.35±0.03); all chemicals were from Sigma, United Kingdom. Left ventricular (LV) trabeculae and atrial pectinate preparations (250–600 µm diameter, 3–5 mm length) were dissected at room temperature. These preparations possess a high degree of cellular alignment15,16 and are ideal for measurement of CV and resistivity in a single, longitudinal axis.

**Human LV Myocardium**

Basal LV septum from 6 patients with obstructive hypertrophic cardiomyopathy undergoing surgical myectomy was obtained. Samples were dissected carefully and the section handled by surgical instruments was immediately excised with a sharp blade and discarded. Samples were placed immediately in Ca2+-free Tyrode solution at room temperature and preparations dissected in the laboratory within 30 minutes.

A portion of the tissue was also frozen immediately in liquid N2 and stored at −80°C to measure cell diameter. Guinea pigs were handled in accordance with Guidance on the Operation of the UK Animals Act (1986). Human tissue was used with approval of the local ethics committees and with informed patient consent.

**Measurement of Cell Diameter**

Frozen, transverse 10-µm sections (cryostat: Thermo Shandon) were mounted on poly-L-lysine–coated glass slides and stained with hematoxylin and eosin. Cell diameters through the nucleus, and the muscle/interstitial cross-sectional area ratio, were measured in $≥10$ muscle/interstitial cross-sectional area ratio, were measured in each preparation cross-section area occupied by muscle. The non-muscle fraction of cross-section area was calculated from the value of $r_c$, assuming it was filled with Tyrode solution (49 Ω·cm$^{-1}$). To determine the effects of carbenoxolone, preparations were pretreated for 30 minutes with Tyrode solution + 20 µmol/L carbenoxolone before mounting in the impedance bath, containing Tyrode solution and carbenoxolone solution in the outer chambers.

**Intracellular Electrophysiological Measurements and Measurement of CV**

Methods have been described previously in detail14; preparations were superfused with Tyrode solution (37°C, 4 mL·min$^{-1}$) in a horizontal trough. Longitudinal CV was measured by stimulating the preparation at 1 end with insulated Ag-AgCl electrodes (10 µs pulses, 1 Hz, 1.5x threshold). APs were recorded at 6 to 10 distances, $d$, >1 mm from the stimulation site to avoid virtual electrode effects at shorter distances. The slope of the relationship between $d$ and the delay, $t_d$, between stimulus artifact and AP upstroke was used to calculate the value of CV; plots were rejected if the $r^2$ values were <0.95. At least 2 separate estimates of CV were made in each preparation and were always within 5% of each other. The time constant of the subthreshold AP foot, $\tau_{ss}$, was calculated from the slope of a semilogarithmic plot of the initial 10 to 12 mV of conducted APs. AP duration was the time from maximum upstroke rate (dV/dt) to 50% or 95% repolarization (APD50, APD95) in ventricle and 75% repolarization (APD75).

**Measurement of Myocardial Impedance, Calculation of GJ Resistivity, and Estimation of Extracellular Resistance**

The method and its validation have been described previously in detail.3 Myocardial preparations were placed in a 3-chambered bath; the outer chambers were superfused with Tyrode solution at 37°C and the muscle in the central chamber was coated with mineral-oil gel. Alternating current (0.02–100 kHz) was passed between platinum (Pt)-black electrode in the outer chambers; current therefore flowed through the intracellular muscle pathway within the oil-gap, with a fraction through a parallel extracellular shunt. System resistance, $r_s$, and capacitance, $c_s$, were recorded with a balanced Wien bridge (Wayne-Kerr, United Kingdom). Total preparation impedance, $z_s$, was modeled as $z_s = (r_s + j\omega c_s)(r_c + j\omega c_c)$, where $r_c$ is the resistance of the extracellular shunt and $z_c$ is the impedance of the intracellular pathway. $r_c$ was measured separately by measuring the resistance between 2 Pt-black needle electrodes a known distance apart in the muscle within the oil-gap. Pt-black electrode resistance, $r_p$, and capacitance, $c_p$, were measured separately in a large volume of Tyrode solution and subtracted from recorded values of $r_s$ and $c_s$.

Longitudinal impedance, $z_c$, was analyzed as 2 series components: sarcomplasm resistance, $r_s$, and GJ impedance, $z_c$ (ie, $z_c = z_c + r_c$). $z_c$ values were expressed as resistance, $r_c$, and reactance, $-j\omega c_c$, components (ie, $z_c = r_c + j\omega c_c$). Plots of $r_c$ versus $-j\omega c_c$ yielded semicircular loci (Figure 1); data were fitted by a circle equation $(r_c - a)^2 + (c_c - b)^2 = c^2$ (a, b, c constants) to the left-hand locus, using data derived from measurements at 1 to 100 kHz and intercepts with the $r_c$-axis (abscissa) estimated. This locus derived from the intracellular pathway, whereas the right-hand locus derives from the surface membrane.4 The high-frequency (left) intercept is a function of $r_c$ and the right-hand intercept a function of $z_c$; junction resistance, $r_c$, was the difference between $z_c$ and $r_c$. Preparation length and radius in the oil-gap were measured. Lower case values of variables ($r_s$, $c_s$: Ω·cm$^{-2}$) were converted to specific ($R, X$: Ω·cm) values by scaling to the proportion of the preparation cross section area occupied by muscle. The non-muscle fraction of cross-section area was calculated from the value of $r_c$, assuming it was filled with Tyrode solution (49 Ω·cm$^{-1}$).

Figure 1. Analysis of intracellular impedance in terms of resistive, $R$, and reactive, $X$, components. The left semicircular dispersion was fitted to a plot of $R_1$ against $-X_s$ at frequencies between 0.02 to 100 kHz using the equation, $X_s = \sqrt{a^2 - (R_s + b)^2} - c$, where a, b, and c are constants. Intercepts on the abscissa are measures of $R_1$ (R1) and R2 (R2).
in atrium. \(\text{dV/dt}_{\text{max}}\) was measured by analogue differentiation of the AP waveform.

After control measurements, continuous intracellular impalements were maintained in Tyrode solution with 20 \(\mu\text{mol/L}\) carbenoxolone for \(\approx 30\) minutes, or until time of maximal conduction delay and measurements made at regular intervals. A washout of carbenoxolone was commenced after a stable delay had been observed for 5 minutes.

**Effects of GJ Uncoupling on Bipolar Electrogram Duration**

The influence of GJ uncoupling on local myocardial activation time was determined from the duration of the bipolar electrogram during electrophysiology studies, as a clinically accessible measure of local microscopic CV recorded from electrodes in contact with myocardium. A quadrupolar mapping catheter, with 2-mm electrode spacing, created electro-anatomic maps of right atrial and right ventricular activation (Carto, Biosense Webster) during sinus rhythm. Electrogram duration (filtered 30–500 Hz) was measured at >20 sites throughout the atrium and ventricle of each patient before and 1 hour after administration of a single oral carbenoxolone dose (100 mg), which reaches 90% of peak concentration (15 \(\mu\text{g/mL}\)) within 40 minutes.\(^2\)

A CARTO mapping system tagged recording sites so that measurements were made at the same locations pre- and postadministration of carbenoxolone. Electrogram duration was measured from the beginning of the first deflection from baseline to return of the last deflection. The study was approved by the local ethics committee.

**Statistics**

Data are mean±SD. Group comparisons were performed using ANOVA with post hoc analysis using Bonferroni test. The null hypothesis was rejected at \(P<0.05\). Linear and nonlinear curve-fits used a least-squares program (KaleidaGraph, Synergy Software) that with either an in-built subroutine for linear fits (Figure 2) or required the experimenter to add the equation of a circle (above) to estimate parameters from the \(-X/R\) plots from impedance data, including initial estimates of the parameters. Pearson correlation coefficients, \(r^2\), were derived for linear fits and significance was calculated by calculation of \(t\) from the relationship: 
\[ t = r / \sqrt{(1-r^2)/(n-2)} \] 
\((n=number\ of\ data\ points)\) and \(P\) calculated for \(n\)–2 degrees of freedom. Coefficients of variation, \(c_v\), were calculated from sample means, \(\bar{X}\), and SD, \(s\), with correction for small sample numbers:
\[ c_v = \frac{s}{\bar{X}} \left(1 + \frac{4}{n}\right), \quad n=number\ of\ samples. \]

**Results**

**Guinea-Pig Myocardium: Impedance Values and AP Propagation Velocity**

Figure 1 shows a plot of resistance, \(R_j\), versus reactance, \(-X\), for a guinea-pig ventricular preparation in the oil-gap chamber. Two dispersions are seen: the partial, low frequency (right-hand) one is attributed to the surface membrane in the 2 outer chambers; the high frequency (left-hand) one to the junction impedance in the intracellular pathway and was analyzed as in Methods.\(^3\) The intercepts of the high-frequency dispersion on the \(R_j\)-axis (\(R_s\) and \(R_j\)) correspond to the sarcoplasmic, \(R_s\), and total intracellular \(R_j\), resistivity respectively: the difference between them is a function of gap junctional resistivity, \(R_j\). Table 1 shows control values of \(R_j\) along with its components \(R_s\) and \(R_j\). \(R_j\) and \(R_s\) values were greater in LV compared with left and right atrial myocardium, \(R_j\) was similar in samples from all 3 chambers. CV values are shown in Table 2; values were significantly smaller in LV compared with left atrium (LA) or right atrium (RA) preparations. The coefficients of variation, \(c_v\), of the sample data were 3% to 10% for \(R_s\), 2% to 12% for CV, and 6% to 20% for \(R_j\) values.

**Effects of Carbenoxolone on Intracellular Impedance**

Table 1 shows that carbenoxolone increased \(R_j\) in all preparations. Examination of the component values of \(R_j\) showed that the increase was solely because of an increase of junction resistivity, \(R_j\); sarcolemmal resistivity, \(R_s\), was not significantly affected. The mean proportional increases of \(R_j\) were not significantly different between the 3 preparations (33±5%, 41±4%, and 32±5%; LV, LA, and RA respectively).

**Effects of Carbenoxolone on Resting and APs**

Table 2 shows that carbenoxolone had no effect on the resting membrane potential, \(E_m\), AP duration or \(\tau\). The increase in \(\text{dV/dt}_{\text{max}}\) with LV and LA preparations (unaffected in RA), and the increase of AP amplitude in LA preparations are consistent with GJ uncoupling causing increased charge

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**Figure 2.** The relationship between conduction velocity (CV) and total intracellular resistivity, \(R_j\) (A) and junction resistivity, \(R_j\) (B). Conduction velocity is expressed as the normalized variable, \(k/\text{CV}^2\) (ie, \(K/\text{CV}^210^3\); see text for details). The solid straight lines were obtained by least-squares analysis for the data from guinea pig (closed symbols) of left ventricle (LV), left atrium (LA), and right atrium (RA) in the absence of presence of carbenoxolone (cbx). The datum point of data from human hypertrophic cardiomyopathy (HCM) is also plotted (open symbol). The dotted lines represent extrapolation of the fitted lines to the abscissa (bottom left) or to the HCM datum point (top right).
accumulation in the cell from which recordings are made, and therefore corroborate this mechanism of action of the carbenoxolone. All significant effects of carbenoxolone were fully reversible after 10 minutes of washout, and there were also no changes on washout to those variables unaffected by carbenoxolone itself. Values of $R_i$ were estimated from 1-dimensional cable theory and were similar to measured values (Table 1).

### Quantitative Relationship Between CV and GJ Resistivity

An important objective of this study was to describe the quantitative relationship between junction resistivity, $R_j$, and AP CV. This addressed the hypothesis that CV is a function of $R_j$ over a range of values that occur under physiological and pathophysiological conditions, and not only when GJ number varies. As a result, the proportion of extracellular space was similar in samples from all 3 chambers (3.3±1.9%, 5.3±1.7%, and 4.7±2.5%: LV, LA, and RA, respectively). Therefore, a plot of $K/CV^2$ as function of $R_j$ (Figure 2A) uses values for $R_j$, CV and $\tau_{ap}$ from guinea-pig LV, LA, and RA preparations in the absence and presence of carbenoxolone (Tables 1 and 2). Values of myocyte radius, $a$, were 12.5±0.9 μm (LV); 6.3±0.8 μm (LA), and 6.2±0.9 μm (RA). The linear relationship ($r^2$=0.956; $P<0.005$) and the intersection near the origin indicate that equation 1 is an excellent description of the relationship between CV and $R_j$ in the absence and presence of carbenoxolone.

Intracellular resistivity, $R_i$, is further modeled as a linear sum of GJ and cytoplasm resistivities ($R_j$ and $R_c$). Thus, a plot of $K/CV^2$ as function of $R_j$ (Table 1) should also yield a linear plot if $R_j$ is similar in all preparations and in the presence and absence of carbenoxolone (Table 1) with an intercept on the $R_j$-axis equal to a value of $R_j$. Figure 2B shows that data were well described by a linear fit ($r^2$=0.946; $P<0.005$) with data extrapolated to the $R_j$-axis with a value of 106 Ω·cm. This is similar to the mean of the measured values of $R_j$ shown in Table 1 (125±4 Ω·cm, SEM of 6 mean values, n=6) and further supports this analytic approach to describe the relationship between CV and $R_j$.

CV is also dependent on the proportion of extracellular space in multicellular preparations. Thus, variation between myocardium from different cardiac chambers or in the presence of carbenoxolone may influence the relationship in Figure 2. Histological data showed that the proportion of extracellular space was similar in samples from all 3 chambers (3.3±1.9%, 5.3±1.7%, and 4.7±2.5%: LV, LA, and RA, respectively) and was unaffected by carbenoxolone. Estimation of extracellular space proportion from impedance experiments also showed no variation between chambers (1.7±1.2%, 2.7±1.0%, and 2.4±1.4%: LV, LA, and RA, respectively).

### Table 1. Values of Total, $R_i$, Junction, $R_j$, and Sarcoplasmic, $R_c$, Resistivities for Ventricular and Atrial Myocardium

<table>
<thead>
<tr>
<th></th>
<th>Intracellular Resistivity, $R_i$, Ω·cm</th>
<th>Junction Resistivity, $R_j$, Ω·cm</th>
<th>Sarcoplasm Resistivity, $R_c$, Ω·cm</th>
</tr>
</thead>
<tbody>
<tr>
<td>LV</td>
<td>525±50*</td>
<td>393±51*</td>
<td>132±13</td>
</tr>
<tr>
<td>LV+cbx</td>
<td>654±43†</td>
<td>522±80†</td>
<td>132±41</td>
</tr>
<tr>
<td>LA</td>
<td>260±7</td>
<td>147±9</td>
<td>113±13</td>
</tr>
<tr>
<td>LA+cbx</td>
<td>331±22†</td>
<td>207±27†</td>
<td>134±18</td>
</tr>
<tr>
<td>RA</td>
<td>220±21</td>
<td>109±21</td>
<td>111±10</td>
</tr>
<tr>
<td>RA+cbx</td>
<td>271±15†</td>
<td>144±14†</td>
<td>127±16</td>
</tr>
</tbody>
</table>

Mean±SD; n=6 for all values. Cbx indicates carbenoxolone; LA, left atrium; LV, left ventricle; RA, right atrium.

*P<0.05 LV vs LA and RA in control.

†P<0.05 cbx vs control.

One-dimensional cable theory defines the relationship between CV and $R_i$ as:

$$CV^2 = \frac{K}{R_i}$$

where the constant of proportionality, $K$, equals $a^2/C_m \tau_{ap}$. Therefore, $K/CV^2$ values were plotted as a function of $R_j$ (Figure 2A) using values for $R_j$, CV and $\tau_{ap}$ from guinea-pig LV, LA, and RA preparations in the absence and presence of carbenoxolone (Tables 1 and 2). Values of myocyte radius, $a$, were 12.5±0.9 μm (LV); 6.3±0.8 μm (LA), and 6.2±0.9 μm (RA). The linear relationship ($r^2$=0.956; $P<0.005$) and the intersection near the origin indicate that equation 1 is an excellent description of the relationship between CV and $R_j$ in the absence and presence of carbenoxolone.

### Table 2. Action Potential Parameters Before (Control), During Carbenoxolone Exposure and on Washout, in Guinea-Pig Preparations From LV, LA, and RA

<table>
<thead>
<tr>
<th></th>
<th>CV, cm/s</th>
<th>$V_{ap}$, mV</th>
<th>$AP_{max}$, mV</th>
<th>APD$_{50}$, ms</th>
<th>APD$_{75}$, ms</th>
<th>APD$_{95}$, ms</th>
<th>$\tau_{ap}$, ms</th>
<th>Vdft$_{ap}$, V/s</th>
</tr>
</thead>
<tbody>
<tr>
<td>LV</td>
<td>70.9±1.3</td>
<td>−89±3</td>
<td>117±5</td>
<td>176±13</td>
<td>217±12</td>
<td>0.28±0.06</td>
<td>224±37</td>
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<tr>
<td>LV+cbx</td>
<td>59.2±3.1*</td>
<td>−87±4</td>
<td>111±7</td>
<td>170±15</td>
<td>214±14</td>
<td>0.28±0.05</td>
<td>307±91*</td>
<td></td>
</tr>
<tr>
<td>LV wash</td>
<td>71.0±1.7</td>
<td>−86±3</td>
<td>108±10</td>
<td>166±12</td>
<td>210±14</td>
<td>0.27±0.04</td>
<td>204±53</td>
<td></td>
</tr>
<tr>
<td>LA</td>
<td>77.6±4.9*</td>
<td>−75±5</td>
<td>105±1</td>
<td>57±5</td>
<td>0.21±0.02</td>
<td>269±11</td>
<td></td>
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</tr>
<tr>
<td>LA+cbx</td>
<td>60.1±6.1*</td>
<td>−81±9</td>
<td>117±4*</td>
<td>56±7</td>
<td>0.26±0.04</td>
<td>347±13</td>
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<tr>
<td>LA wash</td>
<td>77.0±9.0</td>
<td>−77±19</td>
<td>113±9</td>
<td>56±7</td>
<td>0.21±0.03</td>
<td>302±57</td>
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<tr>
<td>RA</td>
<td>77.5±6.4</td>
<td>−71±5</td>
<td>105±3</td>
<td>53±5</td>
<td>0.27±0.07</td>
<td>189±34</td>
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<tr>
<td>RA+cbx</td>
<td>68.9±6.7*</td>
<td>−72±14</td>
<td>110±8</td>
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<tr>
<td>RA wash</td>
<td>77.5±6.4</td>
<td>−79±8</td>
<td>108±6</td>
<td>53±7</td>
<td>0.27±0.05</td>
<td>225±42</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Mean±SD, n=6. $\tau_{ap}$ indicates time constant of the AP foot; $AP_{max}$, action potential amplitude; APD$_{50}$, APD$_{75}$ and APD$_{95}$, action potential duration at 50%, 75% and 95% repolarization, respectively; cbx, carbenoxolone; CV conduction velocity; Vdft$_{ap}$, maximum rate of depolarization during action potential upstroke; LA, left atrium; LV, left ventricle; RA, right atrium; and $V_{ap}$, membrane potential.

*P<0.05 carbenoxolone vs control.
CV and Intracellular Resistivity in Human Myocardium

Having established that the relationship between $R_i$ and CV is similar in both atrial and ventricular guinea-pig myocardium, and with modulation of gap-junctional uncoupling, CV and $R_i$ were measured in human myocardial preparations (Table 3) to determine whether the relationship between CV and $R_i$ is consistent in myocardium from different mammalian species. The human ventricular preparations from patients with hypertrophic cardiomyopathy (Table 3) had higher $R_i$ ($P<0.001$) and slower CV ($P<0.001$), longer APD$_{90}$ and APD$_{ap}$ ($P<0.001$) and lower dV/dr$_{max}$ ($P<0.05$) than guinea-pig ventricular preparations. V$_{ap}$, AP amplitude and $\tau$$_{ap}$ were similar. The single datum point for the K/CV$^2$ versus $R_i$ or $R_j$ (Figure 2A and 2B) corresponds well to the guinea-pig data. Thus, the quantitative fit to the data in Figure 2A and 2B was not extended to these data.

Relationship Between CV and GJ Resistance

Of the factors known to affect conduction, variation of Na$^+$ current density was not a significant contributor as there was no association between CV and either dV/dr$_{max}$ or $\tau$$_{ap}$, both influenced by Na$^+$ current density.$^{23}$ Further, carbenoxolone-induced slowing of conduction produced no significant alterations to AP morphology, other than a small transient increase in left atrial AP amplitude as would be expected with uncoupling. The findings therefore indicate that GJ resistivity, $R_j$, is the main determinant of CV under the conditions of these experiments. The initial data analysis used 1-dimensional cable theory and 2 factors suggested that this provided a good approximation: (1) the similarity of the estimation of $R_i$ (Figure 2B) and the actual value (Table 1); (2) the concordance between the measured values of $R_i$ (Table 1) and those calculated by cable theory. The latter may be used to calculate $R_i$ from the relation $R_i = \frac{\alpha}{2CV^2C_m} \left( \frac{\tau_{ap}}{\tau_m} \right)^{-1}$, $C_m$ and $\tau_m$ have standard values of 0.9 $\mu$F/cm$^2$ and 5 k$\Omega$-cm$^2$, respectively. This yields mean values of 471, 267, and 203 $\Omega$-cm and may be compared with values of 525, 260, and 220 $\Omega$-cm, respectively, as measured by experiment. Several murine Cx43-gene knockout studies showed CV was unaltered by 50% reduction of ventricular Cx43 expression (Cx43$^{-/-}$ heterozygous mice).$^{6-8}$ Only when Cx43 was reduced by $\approx$90% (Cx43$^{-/-}$ homozygous mice) was CV slowed by 50%.$^{24}$ These results contradict both the present investigation and other studies in canine and human myocardium where modest reductions of Cx43 expression resulted in significant conduction slowing.$^{12,13}$ Although transgenic studies have provided invaluable insight into myocardial physiology,

Table 3. Clinical Characteristics and Physiological Parameters of Patients With Hypertrophic Cardiomyopathy

<table>
<thead>
<tr>
<th>Clinical Data</th>
<th>Electrophysiological Data</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>V$_{ap}$, mV</td>
</tr>
<tr>
<td>Gender</td>
<td>AP$_{ap}$, mV</td>
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<tr>
<td>NYHA class</td>
<td>APD$_{90}$, ms</td>
</tr>
<tr>
<td>Max LV wall thickness, mm</td>
<td>APD$_{ap}$, ms</td>
</tr>
<tr>
<td>QRS duration, ms</td>
<td>$\tau$$_{ap}$, ms</td>
</tr>
<tr>
<td>QTC, ms</td>
<td>dV/dr$_{max}$</td>
</tr>
<tr>
<td>LV ejection fraction, %</td>
<td>Cell diameter, $\mu$m</td>
</tr>
<tr>
<td>LV end-diastolic diameter, mm</td>
<td>$\sigma$, cm.s$^{-1}$</td>
</tr>
<tr>
<td>LV end-systolic diameter, mm</td>
<td>$R_j$, $\Omega$-cm</td>
</tr>
<tr>
<td>Fractional shortening, %</td>
<td>$R_j$, $\Omega$-cm</td>
</tr>
<tr>
<td>Resting outflow gradient, mm Hg</td>
<td>$R_j$, $\Omega$-cm</td>
</tr>
</tbody>
</table>

Mean±SD, n=6. AP$_{ap}$ indicates action potential amplitude; APD$_{90}$ and APD$_{ap}$ action potential duration at 50% and 95% repolarization, respectively; F, female; LV, left ventricle; M, male; NYHA, New York Heart Association Classification; and QTC, corrected QT interval.
there remain confounding factors making interpretation of the CV data difficult. These include upregulation of Na current density\textsuperscript{23} and a compensatory increase of intercellular coupling via Cx45 channels,\textsuperscript{26} both of which would attenuate any reduction of CV because of loss of GJs. In the present study, these confounding factors were absent, confirming that $R_j$ is a significant factor of $R_i$, especially in ventricular myocardium. It has also been proposed that sarcoplasmic resistivity, $R_s$, contributes more than $R_j$ to total intracellular resistivity, $R_i$, so that changes to $R_i$ would have a relatively minor influence on $R_j$ and hence CV.\textsuperscript{25,27} However, this study demonstrated that in ventricle $R_i$ is the major contributor to $R_i$ and in atrium contributes equally with $R_j$. Sarcoplasmic resistivity was constant in different cardiac chambers and in the presence and absence of GJ blockers, and similar to the value measured by independent techniques.\textsuperscript{28}

This lends support to our interpretation that CV is a continuous function of GJ resistivity, $R_j$, under physiological conditions and in the presence of gap-junctional uncouplers. This study also provides a more detailed explanation of previous investigations, which showed that CV and total intracellular resistivity, $R_i$, were related over a range of values during conditions such as hypoxia\textsuperscript{26} and ischemia.\textsuperscript{30} Further, our results demonstrate that carbenoxolone-induced conduction slowing was because of effects on GJs rather than on ion channels, in agreement with previous studies in myocardium.\textsuperscript{31,32}

The linearity of the function between CV and $R_j$ also shows that 1-dimensional cable theory is adequate on a millimeter scale to evaluate those factors that determine the value of CV in multicellular preparations, where conduction can be confined to 1 dimension.

### Comparative In Vivo Measurements With Carbenoxolone

Electrogram duration is a manifestation of local microscopic myocardial conduction.\textsuperscript{33,34} We demonstrated carbenoxolone-induced prolongation of the human bipolar electrogram measured from paired electrodes spaced 2 mm apart. The increases in electrogram duration were of a similar order of magnitude to those of conduction delay directly measured in the isolated guinea-pig myocardium. These results indicate that the intact human heart is susceptible to pharmacological uncoupling in a manner similar to isolated preparations. This effect was observed in patients with and without coronary disease in vivo and indicates that the continuous relationship between GJ electric properties and local propagation velocity is independent of a reduced coupling reserve that may exist in disease states.\textsuperscript{28} These findings accord with studies in mice, in which uncoupling both prolonged the bipolar electrogram and slowed macroscopic ventricular CV.\textsuperscript{35}

### Limitations

Although adjacent papillary/pectinate muscles from the same heart were used for the CV and impedance experiments, measurements were not performed on the same actual preparations, and correlations of $R_j$ and CV therefore assume that given their immediate adjacency in the same animal, the 2 preparations have similar electrophysiological properties. Data from 6 hearts (not included) show that CV determination in adjacent ventricular muscles have values within 10\%, and from 10 other hearts impedance values were within 5\% of each other.

Although cable theory provides a suitable model for conduction in geometrically well-defined tissue, such as papillary and pectinate muscle, it has limitations in modeling conduction in tissues possessing discontinuities such as connective tissue layers that are present in hypertrophic cardiomyopathy myocardium. Therefore, caution must also be applied as proportional extracellular space was greater in these preparations that may confound a direct comparison with the CV versus $R_j$ data from guinea-pig tissue. Although excised normal human myocardium is not easily available, the measurement of electrogram duration in intact, relatively normal human hearts provided strongly supportive evidence of this fundamental relationship.

### Conclusions

In intact myocardium there is a continuous relationship between GJ resistivity and CV over a wide range of values such that conduction slowing may occur with modest increases to GJ resistivity. Alterations in cellular coupling of the order that occur naturally can account for variations in electrogram morphology and CV that are of relevance to interpreting clinical measurement and to arrhythmogenic tendency.

### Acknowledgments

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### Disclosures

None.
References

**CLINICAL PERSPECTIVE**

Modification of gap-junctional function is a potential therapeutic target for treating myocardial electromechanical dysfunction and arrhythmia, but studies of knockout mouse models found evidence of substantial redundancy of gap-junctional coupling that could limit the effect of therapeutic agents. We think that this redundancy may not be the case in naturally occurring myocardium and challenged this concept, by correlating measurements of gap junctional resistivity and conduction velocity, each independently measured under conditions of partial pharmacological gap-junctional uncoupling with carbeneoloxone, in both human and guinea-pig atrial and ventricular myocardium. Studies on muscle preparations of normal (nontransgenic) mammalian myocardium demonstrated a continuous relationship between gap-junctional resistivity and conduction velocity. These findings, particularly when taken in conjunction with our previously published clinical study of gap-junctional modulation, do not support the presence of significant redundancy, but do indicate that gap-junctional coupling is a determinant of conductance and conduction velocity over a broad range of values, and is a target for antiarrhythmic therapies.
Relationship Between Gap-Junctional Conductance and Conduction Velocity in Mammalian Myocardium
Paramdeep S. Dhillon, Rosaire Gray, Pipin Kojodojo, Rita Jabr, Rasheda Chowdhury, Christopher H. Fry and Nicholas S. Peters

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