The Relationship between Gap Junction Conductance and Conduction Velocity in Mammalian Myocardium

Running title: Dhillon et al.; Gap junction conductance and conduction velocity

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Abstract:

Background - Gap junction (GJ) 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 GJs. 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 (LV) myocardium from patients with hypertrophic cardiomyopathy and in guinea-pig atrial and ventricular myocardium before and during pharmacological uncoupling with 20 μM 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 LV, left atrium (LA), and right atrium (RA), 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 $p<0.05$ vs. 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 RA [39.7±4.2 to 42.3±4.3ms ($p=0.01$)] and right ventricle [48.1±2.5 to 53.3±5.3ms ($p<0.01$)].

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.

Key words: gap junctions, electrophysiology, conduction velocity, carbenoxolone
Introduction

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

The situation in intact myocardium is less clear. One-dimensional cable theory predicts \( CV \) to vary inversely with the square root of total tissue axial resistance through the tissue, \( R_i \), corroborated in mammalian ventricle by independent measurement of \( R_i \) and \( CV \). Axial resistance has two series components; one from the sarcoplasm, \( R_s \), and the other from GJs, \( R_j \), each significantly contributing to \( R_i \). 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 gap-junction 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 gap junction resistance and \( CV \) has not been systematically determined, in large part because the two 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 relation 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 occurring ranges of coupling and uncoupling in pathological conditions. We used validated methods\(^5,1^4\) to measure \( R_j \) and \( CV \) and their general relationship in guinea-pig myocardium from different cardiac chambers with and without a gap junction blocker, carbenoxolone, and in human ventricular myocardium excised from patients with hypertrophic cardiomyopathy (HCM). We also examined the effects of carbenoxolone on local, sub-millimeter propagation in intact human myocardial propagation as inferred from changes in bipolar electrogram duration during clinical electrophysiology studies to determine how the \textit{ex vivo} findings are manifest in, and may therefore be inferred from, clinical measurements.

\section*{Methods}

\textbf{Preparations. Guinea pig myocardium}

Male Dunkin-Hartley guinea-pigs (400-600 g) were sacrificed and the hearts rapidly excised and immersed in pre-oxygenated Tyrode’s solution containing (mM) NaCl 118, KCl 4.0, NaHCO\textsubscript{3} 24, NaH\textsubscript{2}PO\textsubscript{4} 0.4, MgCl\textsubscript{2} 1.0, CaCl\textsubscript{2} 1.8, glucose 6.1, Na pyruvate 5.0 (pre-gassed with 95\%O\textsubscript{2}/5\%CO\textsubscript{2}, pH 7.35±0.03); all chemicals were from Sigma, UK. Left ventricular trabeculae and atrial pectinate preparations (250-600 \textmu m diameter, 3-5 mm length) were dissected at room temperature. These preparations possess a high degree of cellular alignment\(^{15,16}\) and are ideal for measurement of \( CV \) and resistivity in a single, longitudinal axis.

\textbf{Human left ventricular myocardium}

Basal left ventricular septum from six patients with obstructive HCM undergoing surgical myectomies 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 Ca\(^{2+}\)-free Tyrode’s at room temperature and preparations dissected in the laboratory within 30 minutes.

A portion of the tissue was also immediately frozen in liquid N\(_2\) 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 \(\mu\)m sections (cryostat: Thermo Shandon, USA) were mounted on poly-L-lysine coated glass slides and stained with haematoxylin and eosin. Cell diameter through the nucleus and the muscle/interstitial cross-sectional area ratio was measured in \(\geq 10\) cells per section, from at least five sections per specimen.\(^1\)\(^7\)

**Measurement of myocardial impedance, calculation of gap-junction resistivity and estimation of extracellular resistance**

The method and its validation have previously been described in detail.\(^5\) Myocardial preparations were placed in a three-chambered bath; the outer chambers were superfused with Tyrode’s 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 electrodes 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, \(r\), and capacitance, \(c\), were recorded with a balanced Wien bridge (Wayne-Kerr, UK). Total preparation impedance, \(z\), was modelled as \(z = (z_1 - r_{cc})/(z_1 + r_{cc})\), where \(r_{cc}\) is the resistance of the extracellular shunt and \(z_1\) is the impedance of the intracellular pathway. \(r_{cc}\) was measured separately by measuring the
resistance between two 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’s solution and subtracted from recorded values of \( r \) and \( c \).\(^5\)

Longitudinal impedance, \( z_i \), was analyzed as two series components; sarcoplasm resistance, \( r_c \), and gap junction impedance, \( z_j \) (i.e. \( z_i = z_j + r_c \)). \( z_i \) values were expressed as resistance, \( r_s \), and reactance, \( -x_s \), components (i.e. \( z_i = r_s + jx_s \); \( j = \sqrt{-1} \)). Plots of \( r_s \) vs \( -x_s \) yielded semi-circular loci (see Figure 1); data were fitted by a circle equation \((r_s-a)^2+(x_s-b)^2=c^2\) \((a,b,c\ \text{constants})\) to the left-hand locus, using data derived from measurements at 1-100 kHz and intercepts with the \( r_s \)-axis (abscissa) estimated. This locus represents that derived from the intracellular pathway, whilst the right-hand locus derives from the surface membrane.\(^5\) The high-frequency (left) intercept is a function of \( r_c \) and the right-hand intercept a function of \( z_j \);\(^5\) junction resistance, \( r_j \), was the difference between \( z_i \) and \( r_c \).\(^18,19\) Preparation length and radius in the oil-gap were measured. Lower case values of variables \((r, x, \Omega . \text{cm}^{-1})\) were converted to specific \((R, X, \Omega . \text{cm})\) values by scaling to the proportion of the preparation cross-section area (CSA) occupied by muscle. The non-muscle fraction of CSA was calculated from the value of \( r_{ec} \), assuming it was filled with Tyrode’s \((49 \ \Omega . \text{cm})\).\(^5\) To determine the effects of carbenoxolone, preparations were pre-treated for 30 minutes with Tyrode’s solution + 20 \( \mu \)M carbenoxolone before mounting in the impedance bath, containing Tyrode’s and carbenoxolone solution in the outer chambers.

**Intracellular electrophysiological measurements and measurement of conduction velocity**

Methods have been described previously in detail;\(^14\) preparations were superfused with Tyrode’s solution \((37^\circ \text{C}, 4\text{ml/min}^{-1})\) in a horizontal trough. Longitudinal \( CV \) was measured by stimulating the preparation at one end with insulated Ag-AgCl electrodes \((10 \mu \text{s pulses, 1 Hz, 1.5-times...} \)
threshold). APs were recorded at six to ten 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, \( t \), between stimulus artefact and AP upstroke was used to calculate the value of \( CV \); plots were rejected if the \( r^2 \) values were <0.95. At least two separate estimates of \( CV \) were made in each preparation and were always within 5% of each other. The time constant of the sub-threshold AP foot, \( \tau_{ap} \), was calculated from the slope of a semi-logarithmic plot of the initial 10-12 mV of conducted APs. AP duration was the time from maximum upstroke rate (\( dV/dt_{\text{max}} \)) to 50 or 95% repolarisation (\( \text{APD}_{50} \), \( \text{APD}_{95} \)) in ventricle and 75% repolarisation (\( \text{APD}_{75} \)) in atrium. \( dV/dt_{\text{max}} \) was measured by analogue differentiation of the AP waveform.

After control measurements, continuous intracellular impalements were maintained in Tyrode’s with 20 \( \mu \)M carbenoxolone for up to 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 five minutes.

**Effects of gap-junction uncoupling on bipolar electrogram duration**

The influence of GJ uncoupling on local myocardial activation time was determined from the duration of the bipolar electrogram (EGM) during electrophysiology studies, as a clinically accessible measure of local microscopic \( CV \) recorded from electrodes in contact with myocardium. A quadripolar mapping catheter, with 2 mm electrode spacing, created electro-anatomical maps of right atrial and right ventricular activation (Carto, Biosense Webster) during sinus rhythm. EGM duration (filtered 30-500 Hz) was measured at >20 sites throughout the atrium and ventricle of each patient before and one hour after administration of a single oral carbenoxolone dose (100 mg), which reaches 90% of peak concentration (15 \( \mu \)g.ml\(^{-1} \)) within 40 min.\(^{20} \) A CARTO mapping system tagged recording sites so that measurements were made at the
same locations pre- and post-administration of carbenoxolone. EGM 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’s test. The null hypothesis was rejected at p<0.05. Linear and non-linear curve-fits used a least-squares program (KaleidaGraph, Synergy Software) that with either an in-built sub-routine 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: \( t = r \sqrt{\frac{(1-r^2)(n-2)}{n-2}} \) (\( n \)=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 standard deviation, \( s \), with correction for small sample numbers: \( c_v = \frac{s}{\bar{x}} \left( 1 + \frac{4}{n} \right) \). \( n \)=number of samples.

Results

Guinea-pig myocardium: impedance values and AP propagation velocity

Figure 1 shows a plot of resistance, \( R_s \), vs reactance, -\( X_s \), 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 two outer chambers; the high frequency (left-hand) one to the junction impedance in the intracellular pathway and was analysed as in Methods.\(^5\) The intercepts of the high frequency dispersion on the \( R_s \) axis (\( R_1 \) and \( R_2 \)) correspond to the
sarcoplasmic, $R_c$, and total intracellular $R_t$, resistivity respectively: the difference between the two is a function of junction resistivity, $R_j$. Table 1 shows control values of $R_t$, along with its components $R_j$ and $R_c$. $R_i$ and $R_j$ values were greater in LV compared to left and right atrial myocardium, $R_c$ was similar in samples from all three chambers. $CV$ values are shown in Table 2, values were significantly smaller in LV compared to LA or RA preparations. The coefficients of variation, $c_r$, of the sample data were 3-10% for $R_i$, 2-12% for $CV$, and 6-20% for $R_j$ values.

**The effects of carbenoxolone on intracellular impedance**

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

**The effect of carbenoxolone on resting and action potentials**

Table 2 shows carbenoxolone had no effect on the resting membrane potential, $E_m$, action potential duration or $\tau_{ap}$. The increase in $dV/dt_{max}$ with LV and LA preparations (unaffected in RA), and the increase of AP amplitude in LA preparations are consistent with gap-junction uncoupling causing increased charge 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 ten minutes of washout, and there were also no changes upon washout to those variables unaffected by carbenoxolone itself. Values of $R_i$ were estimated from one-dimensional cable theory and were very similar to measured values (Table 1).
The quantitative relationship between action potential CV and gap-junction resistivity

An important objective of this study was to describe the quantitative relationship between junction resistivity, $R_j$, and action potential $CV$. 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 gap junction number is almost completely abolished, as is the prevailing interpretation of studies on transgenic mice.

$CV$ also depends upon parameters other than $R_j$; most importantly cell radius, $a$, the initial phase of the AP upstroke (exemplified by the time constant, $\tau_{ap}$) and a constant specific membrane capacitance, $C_m$ (1 μF.cm$^{-2}$). Thus, $CV$ values were normalised to allow for influence of $a$ and $\tau_{ap}$. One-dimensional cable theory defines the relationship between $CV$ and $R_i$ as:

$$CV^2 = \frac{K}{R_i}$$  \hspace{1cm} (1)

where the constant of proportionality, $K$, equals $a/2C_m\tau_{ap}$. Therefore, $K/CV^2$ values were plotted as a function of $R_i$ (Figure 2A) using values for $R_i$, $CV$ 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 indicates equation 1 is an excellent description of the relationship between $CV$ and $R_i$ in the absence and presence of carbenoxolone.

Intracellular resistivity, $R_i$, is further modelled as a linear sum of gap junction 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 $R_c$ 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 $R_c$. 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 very similar to the mean of the measured values of $R_c$ shown in Table 1 (125±4 Ω.cm, SEM of six mean values, $n=6$) and further supports this analytical approach to describe the relationship between $CV$ and $R_i$.

Conduction velocity 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 three chambers (3.3±1.9, 5.3±1.7, 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, 2.4±1.4%: LV, LA and RA respectively).

**Action potential $CV$ and intracellular resistivity in human myocardium**

Having established that the relationship between $R_j$ 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 if the relationship between $CV$ and $R_i$ is consistent in myocardium from different mammalian species. The human ventricular preparations from patients with HCM (Table 3) had higher $R_i$ ($p<0.001$) and slower $CV$ ($p<0.001$), longer APD$_{50}$ and APD$_{95}$ ($p<0.001$) and lower $dV/dt_{\text{max}}$ ($p<0.05$) than guinea-pig ventricular preparations. $V_m$, AP amplitude and $\tau_{\text{up}}$, were similar. The single datum point for the $K/CV^2$ vs $R_i$ or $R_j$ (Figures 2A and B) corresponds well to the guinea-pig data. However, the percentage extracellular space, as obtained from impedance experiments was 12.6±2.2% which was significantly larger than guinea-pig data. Thus, the quantitative fit to the data in Figures 2A
and 2B were not extended to these data.

**The effects of carbenoxolone on bipolar EGM duration in the human heart**

Bipolar EGM duration in eleven patients undergoing electrophysiology study (Table 4) were significantly longer in ventricle compared to atrium (48.1±2.5 vs 39.7±4.2 ms). Carbenoxolone significantly prolonged EGM duration in the RV (53.3±5.3 ms, p<0.01), and in the RA (42.3±4.3 ms, p=0.05) indicating microscopic slowing of CV of an order in the RV (electrogram duration +10.6%) and the RA (+7.0%) similar to the changes in the directly measured CVs in the guinea pig ventricular and atrial preparations (Table 1). Carbenoxolone had no effect on EGM amplitude and is consistent with the interpretation that prolongation of the EGM is due to gap-junction uncoupling.

**Discussion**

This study demonstrated a continuous relationship between CV and gap junction resistivity, $R_j$, across different cardiac chambers, in the presence and absence of an uncoupling agent, indicating that $R_j$ is a significant determinant of CV in myocardium. The study design was novel to obtain values for $R_j$ and CV, measured independently in the same preparation. That CV was significantly slowed by moderate increases of $R_j$, indicates there is no threshold of $R_j$ change in influencing CV. This refutes the concept that there is sufficient redundancy of gap-junction coupling that it takes substantial abolition of gap-junction coupling to affect CV.

**The relationship between CV and gap junction 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 $dV/dt_{\text{max}}$ or $\tau_{\text{ap}}$, both influenced by Na$^+$ current density. 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 gap junction resistivity, $R_j$, is the main determinant of $CV$ under the conditions of these experiments.

The initial data analysis used one-dimensional cable theory and two factors suggested that this provided a very good approximation: a) the similarity of the estimation of $R_c$ (Figure 2B) and the actual value (Table 1); b) 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

$$R_i = \frac{a}{2CV^2 C_m \tau_m} \left(1 + \frac{\tau_{ap}}{\tau_m}\right)^{-1},$$

where $C_m$ 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 respectively and may be compared to values of 525, 260 and 220 $\Omega$.cm as measured by experiment. Several murine connexin-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 about 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, there remain confounding factors making interpretation of the $CV$ data difficult. These include: up-regulation of Na$^+$ current density,$^{25}$ and a compensatory increase of intercellular coupling via Cx45 channels,$^{26}$ both of which would attenuate any reduction of $CV$ due to loss of gap junctions. In the present study these confounding factors were absent, confirming that $R_j$ is a significant fraction of $R_i$, especially in ventricular myocardium. It has also been proposed that sarcoplasmic resistivity, $R_c$, contributes more than $R_j$ to total intracellular resistivity, $R_i$, so that changes to $R_j$ would have a relatively minor influence on $R_i$ and hence $CV$. $^{25,27}$ However, this study demonstrated that in ventricle $R_j$ is the major contributor to $R_i$ and in atrium contributes equally with $R_c$.  

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Sarcoplasmic resistivity was constant in different cardiac chambers and in the presence and absence of gap junction blockers, and similar to the value measured by independent techniques.28

This lends support to our interpretation that \( CV \) is a continuous function of gap junction 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,29 and ischaemia.30 Further, our results demonstrate that carbenoxolone-induced conduction slowing was due to effects on gap junctions rather than ion channels, in agreement with previous studies in myocardium.31,32

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

**Comparative in vivo measurements with carbenoxolone**

EGM duration is a manifestation of local microscopic myocardial conduction.33,34 We demonstrated carbenoxolone-induced prolongation of the human bipolar EGM measured from paired electrodes spaced 2 mm apart. The increases in EGM 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 gap-junction electrical properties and local propagation velocity is independent of a reduced coupling reserve that may exist in disease states.28 These findings accord with studies in mice, in which uncoupling both prolonged the bipolar EGM and slowed macroscopic ventricular CV.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 \( R_i \) and \( CV \) therefore assume that given their immediate adjacency in the same animal, the two preparations have similar electrophysiological properties. Data from six hearts (not included) show that \( CV \) determination in adjacent ventricular muscles have values within 10\%, and from ten 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 modelling conduction in tissues possessing discontinuities such as connective tissue layers that are present in HCM myocardium. Therefore caution must also be applied as proportional extracellular space was greater in these preparations that may confound a direct comparison to the \( CV \) vs \( R_i \) 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 gap-junction resistivity and conduction velocity over a wide range of values such that conduction slowing may occur with modest increases to gap junction resistivity. Alterations in cellular coupling of the order that occur naturally can account for variations in electrogram morphology and conduction velocity that are of relevance to interpreting clinical measurement and to arrhythmogenic tendency.
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Conflict of Interest Disclosures: None.

References:


Table 1. Values of total, $R_i$, junction, $R_j$, and sarcoplasmic, $R_c$, resistivities for ventricular and atrial myocardium: Mean±sd; $n=6$ for all values. *$p<0.05$ left ventricle (LV) vs. left atrium (LA) and right atrium (RA) in control; † $p<0.05$ carbenoxolone (cbx) vs. control.

<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>
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<tr>
<td>LV</td>
<td>525±50*</td>
<td>393±51*</td>
<td>132±13</td>
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<td>331±22†</td>
<td>207±27†</td>
<td>134±18†</td>
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<td>RA</td>
<td>220±21</td>
<td>109±21</td>
<td>111±10</td>
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<tr>
<td>RA + cbx</td>
<td>271±15†</td>
<td>144±14†</td>
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Table 2. Action potential parameters before (control), during carbenoxolone (carb) exposure and on washout, in guinea-pig preparations from left ventricle and left & right atrium. Mean data±s.d, n=6, * p<0.05 carbenoxolone vs. control.

<table>
<thead>
<tr>
<th></th>
<th>CV cm/s</th>
<th>V_m mV</th>
<th>AP_amp mV</th>
<th>APD_{50} ms</th>
<th>APD_{75} ms</th>
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<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></td>
<td>0.21±0.02</td>
<td>269±11</td>
<td></td>
</tr>
<tr>
<td>LA + cbx</td>
<td>60.1±6.1*</td>
<td>-81±9</td>
<td>111±4*</td>
<td>56±7</td>
<td></td>
<td>0.26±0.04</td>
<td>347±13</td>
<td></td>
</tr>
<tr>
<td>LA wash</td>
<td>77.0±9.0</td>
<td>-77±19</td>
<td>113±9</td>
<td>56±7</td>
<td></td>
<td>0.21±0.03</td>
<td>302±57</td>
<td></td>
</tr>
<tr>
<td>RA</td>
<td>77.5±6.4</td>
<td>-71±5</td>
<td>105±3</td>
<td>53±5</td>
<td></td>
<td>0.27±0.07</td>
<td>189±34</td>
<td></td>
</tr>
<tr>
<td>RA + cbx</td>
<td>68.9±6.7*</td>
<td>-72±14</td>
<td>110±8</td>
<td>53±4</td>
<td></td>
<td>0.26±0.07</td>
<td>228±56</td>
<td></td>
</tr>
<tr>
<td>RA wash</td>
<td>77.5±6.4</td>
<td>-79±8</td>
<td>108±6</td>
<td>53±7</td>
<td></td>
<td>0.27±0.05</td>
<td>225±42</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: V_m, membrane potential; AP_amp, action potential amplitude; APD_{50}, APD_{75} and APD_{95}, action potential duration at 50, 75 and 90% repolarisation respectively; \( t_{ap} \), time constant of the AP foot; dV/dt_{max}, maximum rate of depolarisation during action potential upstroke; CV conduction velocity.
### Table 3. Clinical characteristics and physiological parameters of HCM patients. Mean data±s.d, \( n=6 \).

<table>
<thead>
<tr>
<th>Clinical data</th>
<th>Electrophysiological data</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years</td>
<td>( V_m, \text{mV} )</td>
</tr>
<tr>
<td>Gender</td>
<td>( \text{AP}_{\text{amp}}, \text{mV} )</td>
</tr>
<tr>
<td>NYHA class</td>
<td>( \text{APD}_{50}, \text{ms} )</td>
</tr>
<tr>
<td>Max LV wall thickness, mm</td>
<td>( \text{APD}_{95}, \text{ms} )</td>
</tr>
<tr>
<td>QRS duration, ms</td>
<td>( \tau_{ap}, \text{ms} )</td>
</tr>
<tr>
<td>QTc, ms</td>
<td>( \frac{dV}{dt_{\text{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>( \theta_e, \text{cm.s}^{-1} )</td>
</tr>
<tr>
<td>LV End-systolic diameter, mm</td>
<td>( R_i, \Omega, \text{cm} )</td>
</tr>
<tr>
<td>Fractional shortening, %</td>
<td>( R_j, \Omega, \text{cm} )</td>
</tr>
<tr>
<td>Resting outflow gradient, mm Hg</td>
<td>( \kappa_c, \Omega, \text{cm} )</td>
</tr>
</tbody>
</table>

NYHA= New York Heart Association Classification; LV= left ventricle; QTc= Corrected QT interval. See also Table 2 for abbreviations.

### Table 4. Clinical characteristics and bipolar electrogram (EGM) data pre- and post-carbenoxolone (cbx). Mean data±s.d, \( n=11 \).

<table>
<thead>
<tr>
<th>Clinical data</th>
<th>Electrogram data</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years</td>
<td>Right atrium, ms</td>
</tr>
<tr>
<td>Sex</td>
<td>Right atrium post cbx, ms</td>
</tr>
<tr>
<td>Coronary Disease</td>
<td>Right ventricular, ms</td>
</tr>
<tr>
<td>Left ventricular ejection fraction, %</td>
<td>Right ventricular post cbx, ms</td>
</tr>
<tr>
<td>Left ventricular diastolic size, mm</td>
<td></td>
</tr>
<tr>
<td>Left atrial diameter, mm</td>
<td></td>
</tr>
</tbody>
</table>

NYHA= New York Heart Association Classification; LV= left ventricle; QTc= Corrected QT interval. See also Table 2 for abbreviations.
Figure Legends:

Figure 1. Analysis of intracellular impedance in terms of resistive, $R_s$ and reactive, $X_s$, components. The left semicircular dispersion was fitted to a plot of $R_s$ 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 $R_s$ (R1) and $R_t$ (R2).

Figure 2. The relationship between conduction velocity (CV) and total intracellular resistivity, $R_t$ (part A) and junction resistivity, $R_j$ (part B). Conduction velocity is expressed as the normalised variable, $k/CV^2$ (i.e. $K/CV^2*10^3$, 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 HCM is also plotted (open symbol). The dotted lines represent extrapolation of the fitted lines to the abscissa (lower left) or to the HCM datum point (upper right).
The Relationship between Gap Junction Conductance and Conduction Velocity in Mammalian Myocardium

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