Interstitial volume modulates the conduction velocity-gap junction relationship

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Veeraraghavan R, Salama ME, Poelzing S. Interstitial volume modulates the conduction velocity-gap junction relationship. Am J Physiol Heart Circ Physiol 302: H278–H286, 2012. First published October 21, 2011; doi:10.1152/ajpheart.00868.2011.—Cardiac conduction through gap junctions is an important determinant of arrhythmia susceptibility. Yet, the relationship between degrees of Gj uncoupling and conduction velocity (θ) remains controversial. Conflicting results in similar experiments are normally attributed to experimental differences. We hypothesized that interstitial volume modulates conduction velocity and its dependence on Gj. Interstitial volume (V_{IS}) was quantified histologically from guinea pig right ventricle. Optical mapping was used to quantify conduction velocity and anisotropy. The results from genetic and pharmacological experiments suggest there may be other factor(s) that modulate the conduction velocity-gap junction (θ-Gj) relationship. Notably, multiple cardiac diseases associated with Gj remodeling (1, 10) are also linked with myocardial edema (7, 28). Yet, little is known about the electrophysiological impact of edema. Therefore, we hypothesized that differences in tissue hydration may modulate the θ-Gj relationship. We demonstrate in Langendorff-perfused guinea pig hearts that the perfusion-dependent changes in interstitial volume (V_{IS}) are a significant modulator of conduction velocity, particularly transverse conduction (θ_T), as well as of the θ-Gj relationship.

METHODS

The investigation conforms to the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996). All animal study protocols were approved by the Institutional Animal Care and Use Committee at the University of Utah.

Western Immunoblotting

Western immunoblotting was performed as described previously (45) to determine protein expression in the ventricles. Total Cx43 and phosphorylated Cx43 (pCx43) were respectively measured using a rabbit anti-Cx43 polyclonal antibody (Invitrogen, Carlsbad, CA) and a rabbit anti-phospho-connexin43 (Ser368) polyclonal antibody (Cell Signaling Technology, Danvers, MA) followed by the goat anti-rabbit HRP-conjugated secondary antibody (JacksonImmuno, West Grove, PA). The blots were then stripped and reprobed for actin as loading control (290.7 ± 23.2 mOsm), compounds demonstrated to reduce myocardial tissue water content (2, 31, 35), for 15 min. The osmolarity of the Tyrode’s solution was significantly higher than control (14.8 ± 4.2 mOsm, respectively), whereas the osmolarity of the mannitol solution was significantly lower than control (14.8 ± 0.1 mOsm, respectively).
electrophysiology was reached within about 10 min of perfusion for both agents as well as for control (after cannulation) and was maintained for 60 min after perfusion (data not shown). All measurements were performed in this 60-min window. The Cx43 uncoupler carbenoxolone was applied in 10–50 µM doses to assess the G-J relationship. Preparations were paced epicardially from the center of either the right or left ventricle (LV) at a basic cycle length (BCL) of 300 ms as described previously (45).

**Guinea Pig Cardiomyocyte Isolation**

Myocytes were isolated from excised, Langendorff-perfused hearts by enzymatic digestion. Hearts were perfused for 8 min with a calcium-free Tyrode’s solution followed by an enzyme cocktail consisting of collagenase type 2 (Worthington Biochemical, Lakewood, NJ) and protease type XIV (Sigma Aldrich, St. Louis, MO) dissolved in Tyrode’s solution containing 0.1 mM calcium for 13 min. The enzyme solution was washed out for 5 min by perfusing 0.1 mM calcium solution. After the washout, the hearts were removed from the cannula and the right and left ventricular free walls were sectioned out and then immersed in 0.1 mM calcium solution. Each piece of tissue was cut into small bits and gently shaken with Tyrode’s solution containing 2% albumin for 10 min to release the myocytes. Myocytes were moved to solutions of increasing calcium concentration (to reach a final concentration of 1 mM in 3 sequential steps) with 6 min allowed for equilibration with each solution.

**Histology**

Computer morphometry was performed, as described previously (13, 40), on hematoxylin and eosin (H&E)-stained transmural slices of gluteraldehyde-fixed ventricular myocardium to quantify VIS. A range of fixatives compared (ethanol, 18% formaldehyde, 27% gluteraldehyde, and 8% gluteraldehyde) based on the uniformity of H&E staining and degree of artifacts/tissue damage (data not shown) and gluteraldehyde was determined to yield the best results. Therefore, gluteraldehyde was used as the fixative in all experiments. A second set of slides was prepared from tissue samples that were frozen and cryosectioned. In brief, the slides were scanned at 0.25 µm/pixel resolution using an Aperio ScanScope XT system (Aperio Technologies, Vista, CA). The interstitial space (IS) was segmented based on eosin staining by color deconvolution (33) using the Aperio ImageScope software (version 9; Aperio Technologies). V\textsubscript{IS} was quantified as the fraction of total tissue area occupied by IS excluding blood and lymph vessels. All measurements were made from the subepicardium, defined as extending from the epicardium to a depth of 500 µm.

**Tissue Water Content**

Total tissue water content was measured by the ratio of the wet weight (WW; measured immediately following perfusion) to the dry weight (DW; measured after drying at 60°C for 24 h), as described previously (21).

**ECG**

A volume-conducted bath ECG was obtained using a silver chloride anode located ~2 cm from lateral wall of the right ventricle (RV) and a similar cathode located ~2 cm from the lateral wall of the LV. ECGs were recorded at 1 kHz and filtered to remove 60 Hz noise.

**Optical Mapping**

Conduction velocity (\(v\)) and anisotropy (AR\(_v\)) were quantified by optical voltage mapping using di-4-ANEPPS (15 µM) as a voltage indicator as described previously (30, 45). In brief, the preparation was stained with di-4-ANEPPS by direct coronary perfusion for 10 min, then excited by three 60 LED light sources (RL5-A9018; Superbrightleds, St. Louis, MO) fitted with 510 ± 5 nm filters (Chroma, Rockingham, VT). Fluoresced light was filtered using a 610 nm LP filter (Newport, Irvine, CA) before being recorded using a SciMedia Micam 02 HS CCD camera (SciMedia, Irvine, CA) in a tandem lens configuration capable of resolving membrane potential changes as small as 2 mV with 1 ms temporal resolution from 90 × 60 sites simultaneously.

Motion was reduced by perfusion of 7.5 mM 2,3-butanediol monoxide. The anterior epicardium was mechanically pressed against the front wall of the perfusion chamber to further stabilize and flatten it, taking care to minimize the pressure applied to the tissue.

Hearts were stimulated with a unipolar silver wire placed on the anterior epicardial surface at the center of the mapping field at 1.5 times the stimulation threshold with a BCL of 300 ms unless otherwise specified. Activation time was defined as the time of the maximum first derivative the action potential as described previously (16). The interpixel resolution was 0.184 mm in the x direction (90 pixels) and 0.199 mm in the y direction (60 pixels).

A parabolic surface was fitted to the activation times as described previously (4). The gradient at each point was assigned a conduction velocity vector. The averaged conduction velocity vectors in the slow and fast axis of propagation (± 15°) are reported since they reflect transverse and longitudinal propagation (17).

Repolarization time (time of full repolarization) was defined as the minimum of the second derivative after the activation time. A low-pass filter was applied to optical action potentials for repolarization time analysis. Action potential duration (APD) was calculated as the difference between repolarization time and activation time. APD\(_{90}\) was calculated as the difference between time to 30% repolarization from peak amplitude and the activation time.
Statistical Analysis

Statistical analysis of the data was performed using a two-tailed Student’s t-test for paired and unpaired data or a single factor ANOVA. The Šidák correction was applied to adjust for multiple comparisons. Fisher’s exact test was used to test differences in nominal data. P < 0.05 was considered statistically significant. All values are reported as mean ± SE unless otherwise noted.

RESULTS

Mechanistic Determinants of Anisotropy

To control for factors known to influence anisotropic conduction (24), we quantified total and \( p\)Cx43 protein expression, myocyte size, and interstitial volume (V\(_{IS}\)). Representative Western immunoblots in Fig. 1, A and B, demonstrate no difference in Cx43 or \( p\)Cx43 band density during normal Tyrode (control) perfusion and after addition of albumin or mannitol to the control solution. The rightmost lane in Fig. 1B is a positive control for \( p\)Cx43 dephosphorylation where the tissue sample was exposed to room temperature for 30 min. Over all experiments, there were no significant changes in either total Cx43 or \( p\)Cx43 expression after perfusion of either albumin or mannitol relative to control (Fig. 1, C and D). On the other hand, RV total Cx43 but not \( p\)Cx43 bands were fainter relative to those from the LV (Fig. 1, A and B). Indeed, overall RV expression of Cx43 but not \( p\)Cx43 was lower relative to LV (Fig. 1, C and D).

Figure 2A provides representative paired wide-field images of a single myocyte exposed to the control, albumin, or mannitol modified Tyrode solution. The difference image represents the digital subtraction of the control from the albumin or mannitol solutions. As can be seen in the representative figure and summary data (Fig. 2, A and B), albumin did not significantly alter cell length or width in isolated myocyte experiments. In contrast, mannitol significantly decreased cell width by 12.1 ± 0.9% and cell length by 1.1 ± 0.4%.

Electrophysiology

Volume-conducted ECGs were recorded from Langendorff-perfused preparations. Representative ECGs in Fig. 4A demonstrate changes in electrophysiology associated with albumin or mannitol perfusion. Specifically, QRS duration was decreased during albumin and prolonged during mannitol perfusion (Fig. 4B). QT interval was not significantly altered in the albumin case, whereas it was prolonged by the same order of magnitude as the QRS interval in the mannitol case (Fig. 4B). QRS and QT were statistically similar between hearts that were optically mapped (pressed against a flat imaging surface) and hearts perfused for Western immunoblotting (freely hanging; data not shown). Importantly, neither intervention was associated with any ST segment changes. Furthermore, neither agent altered action potential duration measured either to full repo-
larization (APD) or to 30% repolarization (APD$_{30}$; Fig. 4, C and D).

**Velocity ($\theta$) and Anisotropy ($AR_{\theta}$) of Conduction**

We next quantified $\theta$ by optical mapping during perfusion of control, albumin, or mannitol modified Tyrode’s solution. Representative optical action potential upstrokes in Fig. 5A demonstrate the expected shorter delay in the longitudinal relative to the transverse direction during control Tyrode perfusion. The representative activation isochrone maps (Fig. 5B) evidence elliptical spread of excitation, consistent with anisotropic conduction. During control conditions, RV transverse ($\theta_{T}$; 21.6 ± 1.3 vs. 17.2 ± 0.8 cm/s; $P < 0.05$) but not longitudinal conduction velocity ($\theta_{L}$; 51.6 ± 2.0 vs. 52.6 ± 1.0 cm/s; $P = $ not significant (NS)) was greater relative to LV. Consequently, anisotropy of conduction ($AR_{\theta}$; defined as $\theta_{L}/\theta_{T}$) was lower in the RV relative to LV (2.4 ± 0.1 vs. 3.1 ± 0.1; $P < 0.05$).

Albumin, which was associated with decreased $V_{IS}$, increased isochrone spacing particularly along the transverse direction, suggesting increased $\theta$ (Fig. 5C). Overall, albumin was associated with increased $\theta_{T}$ (Fig. 6A) and lowered $AR_{\theta}$ (Fig. 6B) in both ventricles relative to control. Conversely, mannitol, which was associated with increased $V_{IS}$, decreased isochrone spacing, also preferentially along the transverse direction, suggesting decreased $\theta$ (Fig. 5D). Overall, mannitol was associated with decreased $\theta_{T}$ (Fig. 6A) and increased $AR_{\theta}$ in both ventricles relative to control (Fig. 6B).

**The $\theta$-$G_{j}$ Relationship**

We probed the effects of perfusate on the $\theta$-$G_{j}$ relationship by measuring $\theta$ and $AR_{\theta}$ in the presence of the $G_{j}$ uncoupler carbenoxolone (32). Under control conditions, there was no significant difference in either $\theta_{T}$ (solid lines) or $\theta_{L}$ (dashed lines) between 0, 10, and 13 $\mu$M carbenoxolone. At 50 $\mu$M, carbenoxolone decreased RV $\theta_{T}$ and $\theta_{L}$ by 25 ± 3 ($P < 0.05$) and 6 ± 3% ($P = $ NS), respectively (Fig. 7A, black lines), increasing $AR_{\theta}$ from 2.0 to 2.5. Similarly, 50 $\mu$M carbenoxolone reduced LV $\theta_{T}$ and $\theta_{L}$ by 19 ± 8 ($P < 0.05$) and 17 ± 3% ($P < 0.05$), respectively (Fig. 7A, black lines). This increased LV $AR_{\theta}$ from 2.5 to 2.7.

During mannitol perfusion, 10 $\mu$M carbenoxolone still did not significantly alter $\theta$ from mannitol alone. However, mannitol + 13 $\mu$M carbenoxolone decreased RV $\theta_{T}$ and $\theta_{L}$ by...
38 ± 9 (P < 0.05) and 16 ± 10% (P = NS) relative to mannitol alone (Fig. 7A, gray lines). This increased RV AR₀ from 3.1 ± 0.2 to 3.5 ± 0.5 (P < 0.05). In the LV, mannitol + 13 μM carbenoxolone decreased θ₁ and θ₁ by 38 ± 8 (P < 0.05) and 7 ± 2% (P = NS), respectively, and increased LV AR₀ from 3.5 ± 0.1 to 4.0 ± 0.3, relative to mannitol alone. Similarly, we measured θ and AR₀ in the presence of varying doses of the mannitol. Interestingly, increasing doses of mannitol were associated with a linear increase in perfusion pressure (R = 0.93; P < 0.05). Under control conditions, 14.3 and 45.3 mOsm mannitol did not significantly slow conduction but 143.2 mOsm mannitol did (Fig. 7B, black lines). These measurements were then repeated in the presence of 15 μM carbenoxolone, which by itself had no significant effect on θ. Carbenoxolone (15 μM) + 14.3 or 45.3 mOsm mannitol significantly slowed conduction relative to 15 μM carbenoxolone alone (Fig. 7B, gray lines). Both ventricles behaved similarly.

**Arrhythmias**

Mannitol by itself increased incidence of spontaneous ventricular tachycardias (VTs; Fig. 8A) compared with control (3 of 8 vs. 0 of 15; P < 0.05). In all cases, the VTs persisted for at least 1 min. There was no incidence of spontaneous VT during albumin perfusion (0 of 7), as during control.

The incidence of spontaneous VTs during perfusion of mannitol + 13 μM carbenoxolone was also higher relative to 13 μM carbenoxolone alone (7 of 9 vs. 0 of 5, P < 0.05; Fig. 8, A and B). Further increasing carbenoxolone dose (15 μM) in the presence of mannitol resulted in loss of capture and conduction failure, which resulted in VT (5 of 5 vs. 0 of 5 with 15 μM carbenoxolone alone, P < 0.05; Fig. 8B).

Finally, 15 μM carbenoxolone + 14.3 mOsm mannitol did not significantly alter VT incidence relative to 15 μM carbenoxolone alone (1 of 6 vs. 0 of 6; P = NS). In contrast, further increasing the mannitol dose (45.3 mOsm) in the presence of 15 μM carbenoxolone significantly increased VT incidence (4 of 6 vs. 0 of 6; P < 0.05) relative to 15 μM carbenoxolone alone.

**DISCUSSION**

Although the role of Gj in cardiac conduction is widely acknowledged, the impact of Gj uncoupling on conduction remains unclear: pharmacological Gj uncoupling has been established to slow conduction (32), yet the impact of pathophysiological Gj remodeling (1, 10) and genetic underexpression of Cx43 (14) remain subjects of debate. We hypothesized that the apparently paradoxical nature of the θ-Gj relationship may be explained by the influence of myocardial tissue architecture. Specifically, we examined the influence of V₁₅ on the
Fig. 6. A: changes in RV, LV transverse conduction velocity ($\theta_T$), and longitudinal conduction velocity ($\theta_L$) from control conditions. Albumin increased RV and LV $\theta_T$ ($^*P < 0.05$ vs. control; $n = 4$). Mannitol decreased RV $\theta_T$ and $\theta_L$ and LV $\theta_T$ ($^*P < 0.05$ vs. control; $n = 4$). Mannitol decreased RV $\theta_T$ and $\theta_L$ and LV $\theta_T$ ($^*P < 0.05$ vs. control; $n = 4$). Mannitol significantly reduced ARs from control by 28.3 and 23.0% in the RV and LV, respectively ($^*P < 0.05$); LV ARs > RV ARs by 24.7% ($^*P < 0.05$). Mannitol significantly increased RV ARs from control by 19.4% ($^*P < 0.05$). RV and LV ARs were no longer significantly different.

Perfusate Effects on Myocardium

Interstitial volume ($V_{IS}$). Albumin, a ~66 kDa globular protein, has been demonstrated to interact with the endothelial glycocalyx and reduce capillary hydraulic conductivity and filtration rate (21, 39, 43). It has been used as a colloidal additive to perfusates during post-ischemic reperfusion to improve vascular barrier function and promote water retention within the vasculature, thereby preventing interstitial edema. In our Langendorff-perfused ventricle preparations (without ischemia), it reduced $V_{IS}$ relative to control (Fig. 3), consistent with previous studies (2, 31).

In contrast, mannitol is a small molecule (molecular weight, 182.17) that raised perfusate osmolarity (see METHODS) and should extravasate freely (22). It has been used as a nonmeatabolizable impermeate added to perfusates during post-ischemic reperfusion to reduces cell edema (i.e., cell swelling) by retaining water outside myocytes (22). In our Langendorff-perfused ventricle preparations, mannitol perfusion was associated with increased $V_{IS}$ (Fig. 3). This may reflect two factors. First, mannitol is a crystalloid impermeate that promotes fluid retention outside myocytes (i.e., in both the interstitium and the vasculature) (22). Finally, in a Langendorff-perfused preparation and unlike an intact animal, the perfusate flow rate is held constant; therefore, changes in total circulating fluid volume and resulting physiological responses, which play a vital role in whole animals, are absent. The combination of these two factors may explain the increase in $V_{IS}$, i.e., interstitial edema observed during mannitol perfusion in our experiments.

Determinants of Measured Cardiac Propagation

Extracellular conductivity. Cable theory, which models conduction in linear, cylindrical strands, predicts that decreasing the conductivity of the extracellular space should decrease cardiac conduction velocity (36). Both albumin and mannitol caused a small but significant decrease in perfusate conductivity (~1.5 mS/cm). However, they had opposite effects on conduction velocity. Therefore, changes in perfusate conductivity do not fully explain our results.

Membrane excitability and electrophysiology. Cellular excitability is also a major determinant of $\theta$, but has not been demonstrated to change ARs (27, 36, 45). Albumin and mannitol preferentially changed $\theta_T$ and thereby ARs, suggesting that cellular excitability may not be the principal mechanism involved. This is further supported by the lack of significant change in APD$_{90}$ or APD or changes in the QT interval beyond the level of changes observed in QRS duration (Fig. 4) during albumin or mannitol perfusion. These data also suggest that the observed effects of albumin and mannitol perfusion are not related to acute adaptation or remodeling as might occur in ischemia (3).
Cell size. The effect of cell size on conduction velocity is still a matter of debate. For example, cable theory predicts that decreased cell width should decrease $\theta$ (36). However, experimental measurements in hypertrophied human ventricular myocardium suggested that decreased cell width may increase $\theta$ (26). Additionally, a recent simulation study of conduction in complex, three-dimensional myocardium suggested that decreasing cell width may preferentially increase $\theta_L$, while also decreasing $\theta_T$ (34). The present study demonstrates that decreased cell width in the presence of mannitol is associated with preferentially decreased $\theta_T$, which demonstrates significant deviation from previous observations. Furthermore, the isolated cell size measurements should be interpreted cautiously since cell size may respond differently to hyposmotic solutions in intact tissue. Further experiments are needed to resolve discrepancies between this and other studies.

Gap junctions. Lower RV Cx43 protein expression relative to LV (Fig. 1) would argue for greater RV AR$_0$, since $\theta_T$ is more sensitive to differences in Gj coupling relative to $\theta_L$ (19, 23). However, the lack of measurable difference in Cx43 suggests similar AR$_0$ between the ventricles. We observed lower RV AR$_0$ relative to LV (Fig. 6), suggesting that Cx43 expression does not fully explain ventricular AR$_0$ differences.

It is possible that increased V$_{IS}$ causes Gj uncoupling due to increased mechanical stress (27). Likewise, decreasing V$_{IS}$ may facilitate Gj formation by bringing cells closer together (8). We measured no change in Cx43 or Cx43 protein levels after perfusion of either albumin or mannitol (Fig. 1). A positive control revealed that we were capable of measuring changes in these parameters, but changes caused by perfusate may have still been below our detection level. Furthermore, although Gj uncoupling has been linked with dephosphorylation, the impact of mechanical disruption of Gj on Cx43 phosphorylation has not been directly explored and requires further investigation.

Technical considerations. It is possible that our interventions may have altered the number of cell layers within the depth from which optical signals are recorded. For instance, decreasing V$_{IS}$ may have increased the number of cell layers from which signals are recorded. This, combined with rotational anisotropy of ventricular myocardium, would increase $\theta_T$ and decrease $\theta_L$. We find that $\theta_T$ and $\theta_L$ both decrease when cells are closer together, arguing against averaging into the depth of tissue as an explanation for our findings. A similar argument can be made for the mannitile case as well.

The mechanical pressure applied to stabilize hearts may have reduced the amount of fluid in the tissue being optically mapped, thereby, leading to an overestimation of the effects of albumin and an underestimation of the effects of mannitol. However, we noted significant changes in QRS duration with both agents, suggesting that the $\theta$ changes we observed optically were occurring globally over the whole ventricles and gentle pressure did not alter QRS or QT relative to free hanging preparations.

Changes in V$_{IS}$ may alter measured $\theta$ by altering myocardial volume, thereby altering the number of cells in the optical field of view. Albumin, which decreased myocardial volume, should bring cells closer together and increase the number of gap junctions per unit distance traveled by the activation wavefront, assuming that intracellular resistivity is unchanged. This in turn would be expected to manifest as apparent conduction slowing (41), whereas albumin had the opposite effect of speeding up conduction. A similar argument can be made for the mannitile case. Therefore, the technical considerations of optical mapping cannot fully explain the data and may require further investigation.

The $\theta$-V$_{IS}$ Relationship

Albumin perfusion was associated with decreased V$_{IS}$, preferentially increased $\theta_T$, and lowered AR$_0$ in both ventricles. Mannitol perfusion, on the other hand, was associated with increased V$_{IS}$ and preferentially decreased $\theta_L$ in both ventricles and increased RV AR$_0$ (Figs. 5 and 6). In short, our results suggest an inverse $\theta$-V$_{IS}$ relationship (decreasing V$_{IS}$ increased $\theta$ and vice versa; Figs. 5 and 6). This is inconsistent with the direct $\theta$-V$_{IS}$ proportionality demonstrated in rabbit papillary muscle (15) and mathematical models (36). There are three plausible explanations for this: as mentioned above, modulating V$_{IS}$ may directly affect gap junctional density at the intercalated disk or the lateral membrane. However, the effects

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**Fig. 8.** A: representative bath ECGs of ventricular tachycardias (VTs) observed during perfusion of mannitol and mannitol + carbenoxolone. B: incidence of spontaneous VTs during perfusion of varying doses of carbenoxolone in the absence (white bars) and presence (black bars) of mannitol (143.2 mOsm). Mannitl significantly increased spontaneous VT incidence relative to control (i.e., 0 mannitol + 0 carbenoxolone) in the presence of 0–15 M carbenoxolone (*$P < 0.05$ vs. control). Furthermore, VT incidence was significantly greater in the presence of mannitol + carbenoxolone relative to carbenoxolone alone for 13 and 15 M doses of carbenoxolone (†$P < 0.05$ vs. corresponding dose of carbenoxolone without mannitol).
we report reached steady state in 10 min, which is faster than the time course of G\textsubscript{i} remodeling reported in acute ischemia (5). This time course may also be shorter than the time required for new G\textsubscript{i} formation (20), especially in intact tissue. Finally, cardiac conduction may involve alternative modes of intercellular coupling that are not exclusively dependent on gap junctions (25, 37). Irrespective of the mechanism, V\textsubscript{IS} plays an important role in modulating conduction.

Notably, V\textsubscript{IS} changes preferentially affected \( \theta \) in the RV relative to the LV (Figs. 5 and 6). Furthermore, when V\textsubscript{IS} was increased AR\textsubscript{\theta} was increased only in the RV (Fig. 6). These data suggest that RV \( \theta \) may be more sensitive to V\textsubscript{IS} changes, possibly as a result of the larger RV V\textsubscript{IS} under control conditions relative to LV (Fig. 3). In other words, the RV may be predisposed to conduction slowing during edema.

**V\textsubscript{IS} Modulates the \( \theta \)-G\textsubscript{j} Relationship and Arrhythmia Incidence**

Experiments using the pharmacological G\textsubscript{j} uncoupler carbene

xolone revealed a nonlinear \( \theta \)-G\textsubscript{j} relationship as reported previously (23). Importantly, our experiments suggest that V\textsubscript{IS} modulates the \( \theta \)-G\textsubscript{j} relationship (Fig. 7A), a notion supported by previous in silico studies (9). Specifically, conduction slowing and spontaneous VTs were observed at lower doses of carbenoxolone in the presence of mannitol compared with control conditions (Figs. 7 and 8). Likewise, 15 \( \mu \)M carbenoxolone, a dose that did not significantly affect conduction, increased conduction sensitivity to V\textsubscript{IS} changes (Fig. 7B); conduction slowing and spontaneous VTs occurred at lower doses of mannitol in the presence of carbenoxolone relative to control. These data suggest an interrelationship between V\textsubscript{IS} and G\textsubscript{j} vis-à-vis conduction.

**Relevance**

These results are important for future studies linking cardiac conduction to G\textsubscript{j} remodeling in disease, because many cardiac pathologies associated with G\textsubscript{j} remodeling (10) also evidence myocardial edema (28). Therefore, interstitial fluid accumulation may constitute an additional proarrhythmic factor that un masks the effects of G\textsubscript{j} remodeling on conduction. Finally, cardiac edema or dehydration may partially explain disparities among previous studies, which have reported on the \( \theta \)-G\textsubscript{j} relationship.

**Conclusion**

In summary, we present here the first demonstration of an inverse relationship between \( \theta \) and V\textsubscript{IS} and that increased V\textsubscript{IS} is associated with slowed conduction, preferentially in the transverse direction. We further demonstrate increased \( \theta \) sensitivity to G\textsubscript{j} uncoupling. Therefore, regardless of the mechanism, this study offers a potential explanation for conflicting results concerning the \( \theta \)-G\textsubscript{j} relationship (6, 14, 18, 29, 38, 41, 42, 44).

**Limitations**

The lack of observable difference in pCx43 protein levels does not necessarily mean that there is no difference or that protein levels correlate directly with the functional Cx43 in channels, particularly given the complex relationship between phosphorylation of Cx43 at various sites and its functionality. We have demonstrated previously that opening outward K\textsuperscript{+} currents slows conduction but in an isotropic manner. Carb

enoxolone may have had offtarget effects; however, 50 \( \mu \)M carbenoxolone (the maximal concentration used in our study) produced no measurable changes in action potential morphology, APD, upstroke velocity (dV/d\textsubscript{max}), and Na\textsuperscript{+}, Ca\textsuperscript{2+}, and K\textsuperscript{+} currents in isolated rabbit ventricular myocytes in a prior study (12). Overall, our data should be interpreted cautiously with the consideration that alternative mechanisms could contribute to the changes of conduction we report. Still, changes in anisotropy have not been associated with trans-sarcolemmal currents, and further studies are required to determine the mechanism by which changing V\textsubscript{IS} modulates AR\textsubscript{\theta} and the \( \theta \)-G\textsubscript{j} relationship.

**DISCLOSURES**

No conflicts of interest, financial or otherwise, are declared by the author(s).

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