Mechanisms of cardiac conduction: a history of revisions

Rengasayee Veeraraghavan,1 Robert G. Gourdie,1,2 and Steven Poelzing1,2

1Virginia Tech Carilion Research Institute, and Center for Heart and Regenerative Medicine, Virginia Polytechnic University, Roanoke, Virginia; and 2School of Biomedical Engineering and Sciences, Virginia Polytechnic University, Blacksburg, Virginia

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Veeraraghavan R, Gourdie RG, Poelzing S. Mechanisms of cardiac conduction: a history of revisions. Am J Physiol Heart Circ Physiol 306: H619–H627, 2014. First published January 10, 2014; doi:10.1152/ajpheart.00760.2013.—Cardiac conduction is the process by which electrical excitation spreads through the heart, triggering individual myocytes to contract in synchrony. Defects in conduction disrupt synchronous activation and are associated with life-threatening arrhythmias in many pathologies. Therefore, it is scarcely surprising that this phenomenon continues to be the subject of active scientific inquiry. Here we provide a brief review of how the conceptual understanding of conduction has evolved over the last century and highlight recent, potentially paradigm-shifting developments.

cardiac conduction; ephaptic coupling; gap junctions; modeling; myocardium

Background

Cardiac conduction is the process by which electrical activation is communicated between myocytes, triggering their synchronous contraction. Impulses originating in the sinoatrial node spread to the atria and via the specialized His-Purkinje conduction system to the ventricles. The sequence of activation thus achieved is key in translating the long-axis contraction of individual myocytes into the complex three-dimensional pumping motion of the heart.

It is well established that aberrant ventricular conduction is associated with a high risk of sudden cardiac death, presumably due to ventricular arrhythmias (67). The prevention of arrhythmias caused by conduction abnormalities remains a topic of intense research in part because there are many factors that are thought to govern cardiac conduction. This review will focus on canonical determinants of conduction: cellular excitability, gap junctions, and tissue architecture in the context of the historical development of the theoretical understanding of conduction in the ventricular myocardium. Additionally, long theorized and new experimental evidence will be discussed concerning alternative modes of action potential propagation from myocyte to myocyte.

The Study of Conduction

Dr. Theodor Wilhelm Engelmann is credited with determining in 1875 that electrical activity in strips of frog atrial muscle spread through muscular tissue (27). It took 39 years before the first direct measurements of cardiac conduction velocity were reported, when Dr. Thomas Lewis and his colleagues first quantified the velocity of cardiac conduction from canine myocardium (61). Conduction velocity is still the primary metric for quantifying the spread of electrical activity in cardiac muscle in part because of its conceptual simplicity and the ease of measuring the time it takes for the electrical wavefront to travel a known distance. More importantly, velocity as a metric of conduction has yielded insights into the mechanisms of electrical activity spread, as conduction velocity is not uniform in all directions from the point of stimulation. This issue of direction-specific conduction spread has provided the foundation of scientific inquiry and debate. But before researchers could even begin to understand the biophysical mechanisms governing cardiac conduction velocity, they first had to determine what caused the action potential, and to do that, the determinants of tissue excitability had to be understood.

Tissue Excitability

The definition of the term “excitability” has evolved with our understanding of the mechanisms underlying this phenomenon. For the purpose of this review, it is convenient to think of excitability in terms of the probability that a propagated action potential will be triggered in response to some quantity of charge entering a cell over a period of time. In neurons, Drs. Wallace Fenn and Doris Cobb (28) suggested in 1936 that a propagated action potential was caused by sodium ions entering an excitable cell and depolarizing the membrane. Subsequently, and perhaps more famously, Drs. Alan Lloyd Hodgkin, Andrew Huxley and Bernard Katz published a series of manuscripts on the topic of neuronal excitation (39–46). Finally, Drs. Alan Lloyd Hodgkin and Paul Horowicz demonstrated that excitability in cardiac myocytes is based on similar mechanisms of sodium entry (38). These early studies, demonstrated that sodium conductance through the cell membrane is very low when the cell is electrically quiescent but increases...
dramatically during the depolarization phase of the action potential. These and other findings prompted Hodgkin and Huxley to suggest that there may be specialized “pores” or channels through which Na⁺ permeates the cell membrane (44). In 1964, Dr. Toshio Narahashi and colleagues reported on the sodium current blocking properties of tetrodotoxin (72), and the use of pharmacological blockers to elucidate properties of the sodium current began in earnest. This line of investigation culminated in the landmark 1976 paper by Drs. Erwin Neher and Bert Sackmann’s in which currents were recorded from single ion channels (73), and finally, the identification of the cardiac isoform of the voltage-gated sodium current (Nav1.5) (30) responsible for depolarizing the preponderance of cardiac myocytes. Overall, it is now well accepted that sodium channel availability is an important determinant of cellular excitability (95).

Potassium channels and excitability. Potassium channels provide outward current that acts to set the resting membrane potential and repolarize the membrane when it is depolarized. Thus these channels shape the action potential after the upstroke, and their relevance to conduction has been thought to be limited to an influence on resting membrane potential. Under pathological conditions such as following ischemia, changes in resting membrane potential are determined by potassium currents, particularly the inward rectifier current (IK1) and the ATP-sensitive potassium current (IKATP). Since the resting membrane potential is a key determinant of sodium channel availability, potassium currents have a significant influence on conduction under such conditions. However, in recent years it has been demonstrated that modulating potassium currents can also affect conduction velocity and its dependence on the sodium current, independent of changes in resting membrane potential: partial inhibition of IK1 can speed up conduction under normal physiological conditions but not when sodium channel availability is compromised (119). On the other hand, pharmacological activation of IKATP and the slow component of the delayed rectifier potassium current (IKs) both slow conduction under normal physiological conditions; however, only the latter slows conduction when sodium channel availability is reduced (118). Overall, these findings suggest that modulating voltage-dependent K⁺ currents affects conduction independent of Na⁺ channel availability, whereas modulating K⁺ currents that do not display voltage-dependent kinetics only affect conduction when Na⁺ channel availability is not reduced.

Furthermore, ongoing studies suggest a codependence between the membrane expression levels of inward rectifier potassium channels (Kir2.1) and sodium channels (Na⁺) (68). An additional line of evidence suggesting an interrelationship between the two channel types comes from the recently identified long QT syndrome type 9, where mutations in the scaffolding protein caveolin-3 was associated with alterations in the biophysical properties of both Kir2.1 and Na⁺ channels. It is therefore likely that potassium channels will continue to be a significant area of research focus in coming years.

Early Revisions and Models of Conduction

Cable theory. In 1952, Dr. Silvio Weidmann demonstrated that electrical communication between cardiac myocytes could be described using a theory first developed in the 19th century by William Thomson (a.k.a. Lord Kelvin) to account for the transmission of electrical signals through a transatlantic telegraph cable (113, 114). Importantly, the application of what is known as cable or the continuous core conductor theory supposes that myocardial tissue is a synctium coupled through purely resistive pathways. While the theory was initially applied to understand action potential propagation through nerves, which are readily conceived as a core of conductive material surrounded by a nonconductive sheath, Weidman extended these treatments to understand electrical impulse propagation through cardiac Purkinje fibers, which are cable-like in structure (124).

Briefly, for a fiber of radius a with intracellular resistance per unit length r_i and extracellular resistance per unit length r_e bounded by a membrane with resistance per unit length r_m and capacitance per unit length c_m, the transmembrane potential v_m can be described by:

$$\frac{r_m}{r_i + r_e} \frac{\partial^2 v_m}{\partial x^2} - c_m \frac{\partial v_m}{\partial t} - v_m = 0$$

By extension, it was demonstrated that conduction velocity is related to r_i and r_e by:

$$\theta = \frac{k}{\sqrt{r_i + r_e}}$$

where k is a constant representing membrane properties (101). In the heart, r_i and r_e are determined by various anatomical structures that compose the electric current path inside, outside, and between myocytes.

Gap junctions. Up until 1954, many theorized cytoplasmic continuity between myocytes, but then Drs. Fritiof S. Sjostrand and Ebba Andersson showed by use of electron microscopy that myocytes are fully bounded by a membrane (96). Therefore, the physical nature of electrical connectivity between cells remained speculative. In the 1960s, Dr. Lloyd Barr demonstrated the existence of low resistance pathways between cardiac myocytes (5, 21), which he termed “the nexus.” These structures have come to be known more widely as “gap junctions,” the name having been coined by Drs. Milton W. Brightman and Thomas S. Reese in 1969 (10).

Since their discovery, gap junctions have been intensely studied, with over 14,000 publications in the literature as of 2013. Each gap junction channel consists of two hexameric connexon hemichannels, each in turn composed of six molecules from the connexin protein family (97). In the heart, three isoforms connexins 40, 43, and 45 are expressed (8, 9, 53, 122) which have different conductances, permeabilities, and cardiac tissue-specific expression patterns (15, 35). Furthermore, different connexin isoforms can combine to form heterotypic and heteromultimeric gap junctions which demonstrate composition-dependent properties (25, 69). However, the situation in mammalian ventricular myocardium is somewhat simplified by the fact that it predominantly expresses connexin 43 (Cx43). Moreover, in the ventricle of humans, and many other mammals, Cx43 is almost exclusively localized to the intercalated disks (34). Thus myocytes are coupled by gap junction channels in primarily end-to-end fashion (49).

Since the majority of early conduction measurements in myocardial tissue were performed macroscopically on Purkinje
fibers, which resemble a cable, it is unsurprising that cable theory continued to fit the available data well. To reconcile the discovery of gap junctions with what appeared to be continuous conduction, many deemed gap junctional conductance to be sufficiently high as to render the cytoplasms of coupled myocytes electrically contiguous (123, 125). Gap junctional conductance was therefore, often incorporated into the intracellular resistance term $r_i$.

Anisotropic conduction. Although thought to be a true syncytium through the first half of the 20th century, ultrastructural studies in the 1950s revealed the cellular nature of cardiac muscle (78, 81, 96). Atrial and ventricular myocardium came to be seen as brick wall-like structures composed of myocytes 100–150 μm long and 10–20 μm wide (19). Therefore, cardiac tissue is anisotropic, with lengthwise orientation of cardiac myocytes and predominantly end-to-end gap junctional coupling (49). This tissue architecture suggests that cardiac conduction should be different parallel to the long axis relative to the short axis of myocytes. Drs. J. Walter Woodbury and Wayne E. Crill showed in the late 1950s that myocardium exhibited a direction-dependent spatial decay of current injected into a point of myocardium, with the longest decay, or space constant, occurring parallel to the long axis of myocytes and the shortest decay occurring perpendicular to myocytes (16). Continuous cable theory was quickly updated to two and three-dimensional models of anisotropic tissue to incorporate the vectors of directional resistivity both inside ($r_i$) and outside ($r_e$) cells (50, 74, 75). From here, these models as a group came to be referred to as “bidomain models” and predicted that cardiac conduction velocity in two- and three-dimensional tissue should be anisotropic. By assuming that cardiac myocardium is a continuous but anisotropic medium, Dr. L. Clerc demonstrated that conduction velocity parallel and transverse to fibers is predicted by an inverse square relationship to total axial resistance, similar to what would be anticipated from cable theory (12). Microelectrode recordings of conduction velocity from multiple sites of myocardial tissue agreed well with these mathematical treatments, and therefore, anisotropic conduction was linked to fiber orientation (22, 93). In this context, it is important to note that the continuous anisotropic resistivity envisaged by bidomain models is a theoretical approximation. In tissue, end-to-end contacts between myocytes can mediate both longitudinal and transverse coupling patterns.

Discontinuous conduction. As technology improved to allow for electrical measurements at greater spatial and temporal resolution, studies in the 1970s revealed yet more complications with the understanding of cardiac conduction. Were the myocardium to behave as a syncytium, the time constant of slow depolarization preceding sodium channel activation ($\tau_{\text{foot}}$) should depend on only axial and membrane resistance and, therefore, be independent of conduction velocity ($\theta$). The model also predicted that the maximal rate of rise of the transmembrane potential ($\frac{dV}{d\theta}$) should depend only on sodium channel availability; therefore, faster conduction should be associated with a larger $\frac{dV}{d\theta}$ (100). However, ground-breaking microscopic measurements made by Dr. Madison Spach and colleagues in both atrial and ventricular myocardium revealed a very different picture: longitudinal conduction, which is faster, was associated with a longer $\tau_{\text{foot}}$ and smaller $\frac{dV}{d\theta}$, relative to slower transverse conduction (105, 106). These experiments began to call into question the use of models based on continuous cable theory to describe anisotropic conduction.

To explain these new discrepancies between measurements made in tissue and those mathematically modeled, the concept of discontinuous conduction was proposed since gap junctions may represent high-resistance pathways between myocytes, rather than the low-resistance structures previously assumed. In fact, direct measurements of gap junctional resistance revealed that resistance at the intercalated disks is approximately equal to the axial resistance of a myocyte (87). Importantly, discontinuous conduction suggested that conduction transverse to the myocyte axis might be slower and also more discontinuous relative to conduction parallel to myocytes, and further experimental evidence supported this assessment (100, 106).

The development of discontinuous conduction theory needed to account for a more complex type of axial resistance that included the following important parameters. First, the mechanisms underlying the resistance of the intercalated disks needed to be explored since the number and function of gap junctions can dramatically affect gap junctional conductance. Second, a conduction wavefront will encounter more discontinuities caused by gap junctions when it travels transverse to myocytes relative to their longitudinal axis, as myocytes are shorter than they are long. Since myocytes organize in a roughly brick-like structure in a sheet or bundle and the orientation of these sheets and bundles maintain a three-dimensional geometry that is optimized for the efficient propulsion of blood, tissue geometry could not be treated as a simple two- or three-dimensional sheet (101). Case in point, myocyte orientation changes from the epicardium to the endocardium (60, 80, 110), and this complex rotational anisotropy has been shown to produce important differences when measuring cardiac conduction (111).

Contemporary Understanding of Anisotropic Conduction

Myocyte geometry. Cardiac myocytes do not maintain the same size and geometry over an organism’s lifetime (63, 103). Changes in cell size and composition can alter cytoplasmic and extracellular resistances in direction-dependent manners, leading to altered conduction anisotropy (92, 106) (the ratio of longitudinal to transverse conduction velocities). Once again, changes in myocyte geometry will also alter the number of gap junctions encountered by the electrical wavefront over a given distance (58, 87, 89). Additionally, cellular hypertrophy over postnatal life is accompanied by changes in GJ localization from uniform distribution around the cell to predominantly intercalated disk localization (3, 36), further confounding the problem of understanding growth-related changes in conduction.

It is noteworthy though that there remain open questions regarding the relationship between cell size and conduction velocity. Some in silico studies have suggested a positive correlation between cell size and conduction velocity (32, 103), whereas experiments in hypertrophied myocardium have suggested a negative correlation between myocyte diameter and conduction velocity (65). A combined experimental and simulation study by Dr. Rob F. Wiegierink and colleagues found increased longitudinal, but not transverse, conduction velocity in failing rabbit hearts; however, the degree of increase was insufficient to compensate for the increased path length.
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resulting from hypertrophy resulting in an increased total activation time (126). To complicate matters further, a recent study by Dr. Thomas Seidel and colleagues suggests that myocyte shape may be as important as myocyte size, if not more so, with respect to modulating conduction velocity in a predictable manner (94).

It continues to be a challenge to understand the role that heterogeneous cell geometry has on conduction in diseases such as cardiac hypertrophic cardiomyopathy (121), because these conditions often also affect the expression and localization of membrane proteins, especially connexins. Additionally, myocyte geometry can also change in the acute time scale in response to ischemia (115) or osmolarity, for example (18).

Nonmyocytes. In addition to cardiac myocytes, the heart also consists of fibroblasts and other nonmyocyte cells. Indeed, these cells outnumber the myocytes although the latter account for the majority of the heart’s volume (128). It has been proposed that fibroblasts could influence cardiac conduction by either acting as passive obstacles or coupling to myocytes and/or each other and acting as either a current sink or even participating actively in conduction. Additionally, under pathophysiological conditions, fibroblasts can transform into myofibroblasts that participate in inflammatory signaling and have also been proposed to modulate conduction (6). However, the extent to which fibroblasts and myofibroblasts electronically couple with myocytes in the ventricular myocardium remains a topic of ongoing inquiry (51, 91, 127). For more detailed discussion of the role of nonmyocytes in conduction, the reader is referred to reviews by Drs. Peter Kohl and (55, 56) and Heather Duffy (6, 23).

In the normal heart, fibroblasts are thought to provide structural support directly, as well as by laying down the extracellular matrix (6). However, under pathophysiological conditions, excessive deposition of extracellular collagen can occur, electrically isolating myocytes from each other, forming barriers to conduction. Indeed, in an elegant study in aging human atrial fiber bundles, Dr. Madison Spach demonstrated how collageneous septa deposited between myocytes create a tortuous electrical path and facilitate reentrant arrhythmias (104). Likewise, in the ventricles, fibrosis can create resistive barriers to conduction and act as an arrhythmogenic substrate. While a certain degree of fibrosis occurs in aging hearts, it can be greatly exacerbated in a variety of pathological conditions (11, 33, 48).

Gap junctions. Given the role gap junctions play in electrically coupling myocytes and because they are remodeled so frequently in cardiac disease, they have received the preponderance of experimental attention. While slowed conduction is well correlated with an increased risk of arrhythmias, there is disagreement in the literature concerning the relationship between the degree of gap junction uncoupling and conduction velocity changes. It is well established that pharmacologically uncoupling gap junctions slow cardiac conduction (57, 87, 88). With this observation comes an important experimental caveat: most articles only report data from doses of gap junction uncouplers that measurably slow conduction (120). This binary data representation therefore excludes the linear correlation between the degree of gap junction uncoupling and conduction slowing, as well as nonspecific (i.e., non-gap junction related) effects of the drugs. Importantly, pharmacological gap junction inhibition studies have provided sufficient evidence that gap junctions at the ends of myocytes constitute the primary source of delay during microscopic conduction (58, 89). Thus modulating gap junctional coupling preferentially impacts transverse conduction velocity, leading to altered anisotropy (90, 92, 106).

Contrast pharmacological gap junction manipulation with genetically manipulating gap junction functional expression. There are a variety of studies on cardiac conduction using the same transgenic mouse lineage expressing 50% of the wild-type levels of Cx43, the primary ventricular gap junction protein. Some groups have reported significantly slowed conduction in mice with a 50% reduction in Cx43 expression relative to wild-type mice (26, 37), whereas others could not measure a difference between them (7, 17, 71, 109, 112, 116, 117).

These very basic studies are critically important to understanding cardiac arrhythmias because gap junction remodeling is a hallmark of cardiac disease. To make matters worse, in disease, the relationship between gap junction remodeling and conduction is even less clear. For example, a reduction of total Cx43 expression in a canine pacing-induced heart failure model was associated with aberrant ventricular conduction and increased arrhythmogenesis (79). In a similar model, gap junction remodeling (relocation) and conduction slowing preceded loss of Cx43 expression (2).

The phrase “gap junctional remodeling” is a catchall term to describe connexin redistribution at the cellular level, posttranslational modification, and total protein expression changes. While it has been demonstrated that cellular redistribution of connexins to the lateral membrane is associated with altered conduction, in hypertrophy, for example, lateralization occurs concomitantly with changes in myocyte geometry (102). Whether or not lateralized Cx43 in the pathological myocardium forms functional gap junctions remains an important and complex question (24). The connexin life cycle is also dynamically regulated by a variety of biochemical pathways including phosphorylation and dephosphorylation of specific amino acid residues of the channel (4, 82, 98, 99). Therefore, gap junctions do not exist in isolation and are part of larger macromolecular complexes and biological pathways.

Dynamic determinants of conduction. In addition to the structural substrate, conduction is also modulated by dynamic functional changes. Primarily, these dynamics result from the interplay between the strength of the excitatory impulse (the source) and the electrical load represented by the tissue it must drive. For example, the electrical load represented by the tissue it must drive (the sink). In the adult canine ventricular myocardium, each myocyte is coupled to an average of 11 ± 3 other myocytes (92). As an activation wavefront spreads through the myocardium, the amount of source available per unit mass of tissue is determined by its excitability, whereas the balance between source and sink is determined by the curvature of the wavefront and its interaction with the architecture of the myocardium: intercellular coupling, fiber orientation, rotational anisotropy, branching tissue geometry, etc. (58, 88, 92, 106). Since local excitability in tissue is dynamically modulated by changes in the shape and duration of action potentials, mismatch between source and sink can arise locally and dynamically, creating a functional substrate for arrhythmogenic conduction defects. Pathophysiological gap junction remodeling and fibrosis can exacerbate source-sink mismatch and thereby the propensity for arrhythmias. For a more detailed
ion channels at the intercalated disk: functional implications

The major impetus for reassessing our understanding of conduction arises from structural insights into the subcellular localization of ion channels. In 1996, Dr. Sidney A. Cohen published the first immunofluorescence images of rat tetradotoxin-resistant sodium channels (rH1), demonstrating their strong localization at the intercalated disk of cardiac myocytes (13). While the importance of ion channels at the intercalated disk has been long postulated as a mechanism of non-gap junction-mediated coupling, this work was mostly the domain of mathematical models (14, 59, 66, 107, 108, 129). These models envision intercellular coupling as occurring thusly: a depolarized myocyte withdraws sodium ions from the restricted junctional cleft via its intercalated disk-localized Na\(_{v1.5}\) channels (Fig. 1A). The resulting depletion of positive charge from the junctional cleft would render the local extracellular potential more negative. Consequently, the transmembrane potential across the apposed membrane of the neighboring myocyte becomes more positive, causing the activation of Na\(_{v1.5}\) channels (Fig. 1B). Thus electrical activation is communicated from one cell to another without the direct transfer of ions between them (Fig. 1C).

Dr. Nicholas Sperelakis is perhaps best known for championing these mechanisms, which he summarized in a 2002 review (108). Later that year, Drs. Jan P. Kucera, Stephan Rohr, and Yoram Rudy confirmed the presence of both sodium channels and connexins at the intercalated disk (59). More importantly, they revised mathematical models of cardiac conduction in a one-dimensional strand, demonstrating that the high density of sodium channels at the intercalated disk could impact cardiac conduction in previously unappreciated ways. Specifically, they concluded that gap junctions are still likely the principal mechanism of electrical transmission between cells, but sodium channels at the intercalated disk could modulate the conduction velocity, gap junction relationship particularly if the space between the myocytes were very small and densely packed with sodium channels.

Since then much evidence has been uncovered to support the existence at the intercalated disk of a macromolecular complex containing the gap junction protein Cx43, as well as cardiac sodium channels (Na\(_{v1.5}\)). Indeed, Cx43 and Na\(_{v1.5}\) were found to coimmunoprecipitate from mouse heart lysates (64) and more recently to colocalize at the intercalated disk (76). Work from the group of Dr. Mario Delmar has demonstrated that mechanical adhesion proteins first localize to sites of cell-cell contact followed by recruitment of Cx43 gap junctions and ankyrin-G, a submembrane adapter protein involved in localizing cardiac sodium channels (Na\(_{v1.5}\)) in the membrane (31). Recent results from the laboratory of Dr. Mario Delmar demonstrate a loss of Na\(_{v1.5}\) from the membrane in conditional Cx43 knockout mice (52) and even suggest that Cx43 is important for the recruitment of Na\(_{v1.5}\) channels into the membrane at the intercalated disk (1, 20).

In addition to sodium channels, evidence has also emerged placing various potassium channel isoforms at the intercalated disk, specifically, the inward rectifier potassium channel (K\(_{IR}\)) (68), the ATP-sensitive potassium channel (K\(_{ATP}\)) (47), the delayed rectifier potassium channel [K\(_{DRA}\)] (83) and the “rapid” delayed rectifier potassium channel [K\(_{DRA}\)] (130). As previously discussed, potassium channels can have significant effects on conduction. Additionally, being localized at the intercalated disk, they could also play a role in intercellular coupling via a potassium-mediated ephaptic mechanism. Briefly, potassium efflux from a depolarized myocyte could lead to a transient accumulation of potassium in the narrow junctional cleft, causing the membrane of the neighboring myocyte to depolarize via an inward potassium current (108).

Although the presence of ion channels at the intercalated disk is suggestive, the ephaptic coupling hypothesis has yet to be experimentally tested in the heart. A key missing element has been the identification of a well-defined structure that could serve as a functional unit of ephaptic coupling, an ephapse. The localization of ion channels at the intercalated disk could have important implications in this regard given the necessity of close apposition between membranes of adjacent cells for ephaptic coupling (62, 70). Taking the experimental evidence together with predictions made by the models, intermembrane spacing could be a key variable in identifying specific microdo-

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**Fig. 1.** Schematic cartoon illustrating the mechanism of ephaptic coupling. A: sodium channels (shown in red) on the depolarized (green) myocyte’s membrane activate and withdraw sodium ions (Na\(^{+}\)) from the restricted extracellular cleft at the intercalated disk. As a result, the transmembrane potential (\(V_{m1}\)) of the first myocyte is elevated. B: concomitant depletion of positive charge from the extracellular cleft lowers the local extracellular potential (\(\Phi_{ex}\)). This leads to an increase in the second, resting (red) myocyte’s transmembrane potential (\(V_{m2}\)), defined as the difference between its intracellular potential and the extracellular potential (\(\Phi_{ex}\)). In turn, sodium channels located at or near the intercalated disk of the second myocyte activate. C: sodium enters the second myocyte via these channels further depolarizing it and triggering an action potential. Thus activation is communicated ephaptically from cell to cell without the direct transfer of ions between them.
mains within the intercalated disk that could function as an ephapse.

**Interstitial volume.** As with any electrical circuit, one must not only consider electrical conduction “forward” through the circuit, but also how the circuit is completed by a current return path. In tissue, the return path can be the interstitial space between the myocytes. At this point, it is useful to revisit cable theory for a moment, as there is no other mathematical theory that has been applied so rigorously to cardiac conduction. Since myocyte geometry is anisotropic, the interstitial space outside the cells is also anisotropic and can affect axial resistance and thereby conduction in an anisotropic manner (77, 101). Cable theory-based models predict a direct proportionality between the volume of the extracellular space and conduction velocity (32, 81), and this has been experimentally supported in the cable-like papillary muscle (29).

However, one of our recent manuscripts provides evidence that modulating the extracellular volume in a heart changes epicardial conduction in a manner inconsistent with bidomain models of cardiac conduction that lack ephaptic coupling (120). Additionally, we demonstrated that small degrees of gap junction uncoupling that did not alter conduction normally, significantly slowed conduction when the interstitial space was increased. This study suggested that the effects of small degrees of gap junctional uncoupling on cardiac conduction can be unmasked by increasing the interstitial volume. What remains unknown though is how increasing interstitial volume produces changes in cardiac conduction inconsistent with bidomain models.

As mentioned earlier, non-gap junction-mediated coupling or “ephaptic” coupling had been proposed by mathematical models, and the distance between cells at the intercalated disk might be an important factor for mediating this type of coupling. A candidate structure that could serve as a functional ephaptic unit as definitive as a synapse or gap junction emerges from recent work by an author of this review together with his colleague Dr. J. Matthew Rhett. In these studies a new feature of cardiac ultrastructure was described: the perinexus, a juxta-gap junction membrane microdomain rich in undocked connexin hemichannels wherein interaction between Cx43 and the cardiac sodium channel (Na\(_\text{v}1.5\)) occurs. Given the close (0–20 nm) apposition of membranes from adjacent myocytes in the vicinity of the gap junction, the perinexus emerges as a strong candidate structure for the cardiac ephapse (84 – 86).

As it stands, there is evidence from both experiments and mathematical models to suggest that ephaptic coupling could be important to cardiac conduction. While previously viewed as a possible alternative to electrotonic coupling, ephaptic coupling has since come to be viewed as operating in tandem with gap junctions, helping sustain conduction when gap junctional coupling is compromised. To fully appreciate the role of ephaptic coupling, a multipronged strategy will be necessary: 1) further whole heart experiments will need to be performed to generate additional examples of complex conduction; 2) the biochemical and functional ultrastructure of the ephaptic machinery will need to be dissected by high-resolution microscopic, molecular, and physiological methods; and 3) multidimensional mathematical models will need to be revised to incorporate ephaptic coupling and tested against the new experimental data.

**Conduction: A New Multifactorial Understanding**

Like all science, the understanding of cardiac conduction has undergone revisions and refinements over the last 130 years, and it appears that discoveries will only continue to accelerate. The picture that is emerging though is that cardiac conduction is not a simple phenomenon mechanistically determined by a few independent biophysical parameters. Rather, cellular excitability, gap junctional conductance, cell size, gap junction localization, subcellular architecture, and ion channel localization are important and interrelated determinants of the conduction phenomenon. While these factors were initially studied vis-à-vis conduction in isolation using a reductionist approach, we are beginning to appreciate that changes in one determinant can potentiate the effects of altering another. This type of higher-level understanding of conduction is critical for determining why certain therapies developed to treat conduction defects are sometimes ineffective or even produce deleterious effects. While it could be argued the interrelated nature and complexity of biological processes means that we will never completely prevent conduction-related arrhythmias, we would suggest that understanding the myriad factors underpinning conduction could eventually provide individualized therapeutic targets that might provide for improved treatment of patients suffering from disease of the heart.

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**DISCLOSURES**

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**AUTHOR CONTRIBUTIONS**

R.V. and S.P. drafted manuscript; R.V., R.G., and S.P. edited and revised manuscript; R.V., R.G., and S.P. approved final version of manuscript.

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