Published in final edited form as:

Cell Commun Adhes. 2014 June ; 21(3): 161–167. doi:10.3109/15419061.2014.905932.

Intercellular Electrical Communication in the Heart: A New, Active Role for the Intercalated Disk

Rengasayee Veeraraghavan, Steven Poelzing, and Robert G. Gourdie

Center for Cardiovascular and Regenerative Biology, Virginia Tech Carilion Research Institute, Roanoke, VA, USA

Abstract

Cardiac conduction is the propagation of electrical excitation through the heart and is responsible for triggering individual myocytes to contract in synchrony. Canonically, this process has been thought to occur electrotonically, by means of direct flow of ions from cell to cell. The intercalated disk (ID), the site of contact between adjacent myocytes, has been viewed as a structure composed of mechanical junctions that stabilize the apposition of cell membranes and gap junctions which constitute low resistance pathways between cells. However, emerging evidence suggests a more active role for structures within the ID in mediating intercellular electrical communication by means of non-canonical ephaptic mechanisms. This review will discuss the role of the ID in the context of the canonical, electrotonic view of conduction and highlight new, emerging possibilities of its playing a more active role in ephaptic coupling between cardiac myocytes.

Keywords

perinexus; gap junction; connexin; sodium channel; $\text{Na}_v1.5$; ephaptic; intercalated disk; conduction

Cardiac conduction is the propagation of electrical excitation through the heart, triggering synchronous contraction of individual cardiac myocytes in order to produce the pulsatile pumping action of the heart. The phenomenon was first demonstrated in preparations derived from frog hearts by Theodor Engelmann in 1874 (Engelmann, 1875). Since then, significant advances have been made in the study of conduction motivated by the role played by abnormal conduction in mediating life-threatening arrhythmias in a variety of disease states.

WHAT IS CONDUCTION?

Cardiac conduction is the electrical phenomenon that integrates the function of individual myocytes to produce the function of the organ as a whole. The cardiac myocyte, which is an electrically active, mechanically contractile cell, is the structural and functional unit of

Address correspondence to Rengasayee Veeraraghavan, Center for Cardiovascular and Regenerative Biology, Virginia Tech Carilion Research Institute, 2 Riverside Circle, Roanoke, VA 24016, USA. Tel: +(801) 448-4249. saiv@vt.edu.

Declaration of interest: The authors report no declarations of interest. The authors alone are responsible for the content and writing of the paper.

cardiac muscle. At rest, the interior of the cardiac myocyte is electronegative with respect to the outside due to the heterogeneous distribution of ions across its membrane, resulting in a negative resting membrane potential. The cell can become electrically excited via rapid influx of positive charge in the form of sodium and calcium ions before being returned to resting potential by the efflux of positive charge in the form of potassium ions. This transient change in membrane potential comprising depolarization and repolarization is termed as action potential. Importantly, electrical activation of the myocytes elicits the release of calcium from intracellular stores, triggering mechanical contraction of the cell. Likewise, repolarization is accompanied by a decline in cytosolic calcium levels, resulting in relaxation of the myocyte. Thus, the individual myocyte functions as an electrically actuated contractile element. In the heart, electrical impulses originating in the pacemaking tissues of the sinoatrial node are communicated through the working myocardium in order to trigger coordinated contraction of individual myocytes, thus achieving the pumping action of the organ as a whole.

Here, we will focus on the mechanisms underlying the propagation of action potentials through the ventricular myocardium. Historically, ventricular conduction has been considered to have two separate aspects: cellular excitability, or the electrical response of a myocyte to a given excitatory impulse, and intercellular coupling. The intercalated disk (ID), which is the primary site of both mechanical and electrical contact has canonically been viewed as being critical to the latter with gap junctions located at the ID thought to afford direct electrical contact between adjacent myocytes. Here, we will review recent structural and functional insights that suggest a much more active role for the ID in conduction including potentially mediating long-theorized alternative modes of action potential propagation from myocyte to myocyte.

ELECTROTONIC CONDUCTION: THE CANONICAL VIEW

Historical perspective

After Engelmann's discovery of the phenomenon in 1874, a major milestone in the study of conduction at the tissue level came in 1914 when Lewis and colleagues reported the first measurements of conduction velocity (Lewis et al., 1914). A major breakthrough at the cellular level followed in 1959 when Hodgkin and Horowicz demonstrated that sodium entry underlay cardiac myocyte excitability (Hodgkin & Horowicz, 1959). They demonstrated a rapid increase in sodium conductance through the cell membrane during the depolarizing phase of the action potential, prompting Hodgkin and Huxley to hypothesize the existence of specialized "pores" or channels that convey Na^+ across the cell membrane (Hodgkin & Huxley, 1952). These "pores" have since then been identified as voltage-gated sodium channels ($\text{Na}_v1.5$) and their role in conduction extensively studied. While these advances occurred in the understanding of cellular excitability, similar strides were being made in explaining intercellular electrical communication. It was theorized early on that the cytoplasm of adjacent myocytes may be contiguous; however, electron microscopy revealed that myocytes are bounded by a membrane (Sjöstrand & Andersson, 1954). Shortly thereafter, Lloyd Barr published electron micrographs of fused membrane structures, which he labeled 'the nexus', connecting adjacent myocytes (Barr et al., 1965; Dewey & Barr,

1962) while Van Der Kloot and Dane proposed that myocytes may be electrically coupled at the ID (Vanderkloot & Dane, 1964). Subsequently, work Revel and Karnovsky demonstrated that 'the nexus' consists of the membranes of adjacent myocytes very closely apposed but nonetheless separated by a narrow gap and coined the term 'gap junction' to describe this structure (Revel & Karnovsky, 1967).

Cable theory

Even as inroads were made into understanding the structural basis of conduction, a conceptual framework began to develop with Silvio Weidman's application of continuous cable theory to describe the electrical properties of cardiac Purkinje fibers (Weidmann, 1952). This approach, later extended to conduction through the ventricular myocardium, envisaged myocytes, electrically connected end-to-end and bounded by highly resistive lipid membranes, as analogous to a jacketed electrical cable consisting of a highly conductive core enveloped in an insulating sheath. Per this model, conduction occurs electrotonically by the movement of ions from cell to cell and the newly identified gap junctions offered an explanation of how. Early on, gap junctions were thought to be of sufficiently low resistance as to render the cytoplasm of coupled cells electrically contiguous and thereby to render cardiac muscle an electrical syncytium. Based on these assumptions, the model predicted an inverse square relationship between conduction velocity and the axial resistance of tissue and experimental measurements made in the late 1950s were consistent with this prediction (Draper & Mya-Tu, 1959; Sano et al., 1959).

Also by the mid twentieth century, cardiac muscle was recognized as being anisotropic composed of brick-like myocytes, 100–150 μm long and 10–20 μm wide, organized in end-to-end fashion (De Mello, 1972). This structural picture fits well with Woddbury and Crill's work in the 1950s demonstrating direction-dependent current decay in cardiac muscle. By extending continuous cable theory to 2- and 3-dimensional models of anisotropic tissue, (Jack et al., 1975; Peskoff, 1979a, 1979b) it was predicted that cardiac conduction velocity in tissue should be anisotropic. Experimental measurements in trabecular muscle as well as myocardial tissue demonstrated faster conduction parallel to fibers than transverse to them, as predicted by cable theory (Draper & Mya-Tu, 1959; Sano et al., 1959; Clerc, 1976).

Gap junctions and conduction

Since first being identified, gap junctions have been the subject of intense study. Composed of proteins from the connexin family, gap junctions not only electrically couple myocytes but also allow the passage of molecules up to 1000 Daltons in size between them. Each gap-junction channel consists of two hemichannels, one from each of the abutting cells, docked together at the site of intercellular contact. In turn, each hemichannel or connexon is a hexamer composed of connexin proteins (Söhl & Willecke, 2004). Three connexin isoforms 40, 43 and 45, so named after their molecular weights and possessing different conductances and permeabilities, are expressed in the heart (Beyer, 1990; Beyer et al., 1987; Kanter et al., 1992; Vozzi et al., 1999) with connexin43 (Cx43) being the principal gap-junction protein in the ventricular myocardium (Coppin et al., 1998; Gourdie et al., 1993). Moreover, in the ventricle of humans, and many other mammals, Cx43 is almost exclusively localized to the

IDs (Gourdie et al., 1991). Thus, gap junctions would appear to afford the type of intercellular electrical conductivity envisioned by cable theory.

A major evolution in our understanding of conduction occurred in the 1970s, when Spach and colleagues performed elegant microscopic measurements of conduction velocity and action potential upstroke demonstrating conduction to be discontinuous. This suggested that gap junctions may represent discontinuities in axial resistance, that is high resistance electrical pathways between myocytes, rather than rendering them syncytial. Indeed, direct measurements revealed the resistance at the ID to be comparable to the axial resistance of the rest of the myocyte (Rohr, 2004). Importantly, cable theory, when updated to include discontinuous axial resistance suggested that transverse conduction might be slower and also more discontinuous than longitudinal conduction and experimental studies proved consistent with this prediction (Spach, 2003; Spach et al., 1981).

For a more detailed discussion of electrotonic conduction, the reader is referred to our recent review (Veeraraghavan et al., 2014) as well as the exhaustive review on the topic by Kléber and Rudy (2004).

BEYOND ELECTROTONIC CONDUCTION

Open questions

Motivation for research into the role played by gap junctions in conduction has stemmed primarily from their being implicated in arrhythmogenic conduction defects in a wide range of diseases. One of the most important open questions in this context is the precise correlation between the degree of loss of gap-junction coupling and the degree of conduction slowing resulting from it. While pharmacological gap-junction uncoupling has been uniformly correlated with slowed conduction, (Rohr, 2004; Kojodjojo et al., 2006; Rohr et al., 1998) the impact of genetically reduced functional expression and pathophysiological remodeling of gap junctions remains to be understood. Among the host of studies conducted on the same transgenic mouse lineage with a 50% loss of Cx43 levels, some groups observed significantly slowed conduction in the mutant mice relative to their wild-type littermates, (Eloff et al., 2001; Guerrero et al., 1997) while others did not (Beauchamp et al., 2004; Danik et al., 2004; Morley et al., 1999; Stein et al., 2009; Thomas et al., 2003; Vaidya et al., 2001; van Rijen et al., 2004). Likewise, reduced Cx43 expression was correlated with arrhythmogenic conduction slowing in a pacing-induced canine heart-failure model, (Poelzing et al., 2004) whereas in a similar model, gap-junction remodeling and loss of Cx43 preceded conduction slowing (Akar et al., 2007).

On the one hand, these conflicting studies hint at the complexity of the physiology underlying the catch-all term ‘gap-junction uncoupling’. Because of the short half-life of Cx43, its functional expression is regulated in a highly dynamic fashion. Further, modulation of Cx43 function is accomplished by post-translational modification, primarily phosphorylation/dephosphorylation of various residues on the molecule’s C-terminus. The mechanisms by which Cx43 expression and function are regulated have been the subject of extensive research and are summarized in reviews by Solan and Lampe (2007, 2014).

Another, more interesting possibility is that the seemingly disparate results vis-à-vis the relationship between gap-junction function and conduction are reflective of non-electrotonic mechanisms involved in cardiac conduction. The impetus to consider this hypothesis derives from investigations of conduction dependence on interstitial volume. If we consider conduction as that occurring via the electrotonic flow of current from cell to cell, then there must be a return path for the current via the interstitial space in order to complete the circuit. Cable theory predicts that the larger the interstitial space, the smaller its axial resistance and therefore, the faster conduction should be (Plonsey & Barr, 2007; Spach et al., 2004). This direct proportionality between interstitial volume and conduction velocity has been experimentally supported in the cable-like papillary muscle (Fleischhauer et al., 1995). However, in a recent experimental study in ventricular myocardium, we demonstrated conduction slowing and increased sensitivity to gap-junction uncoupling in response to an acute increase in interstitial volume (Veeraraghavan et al., 2012). These results suggest that a purely electrotonic model may be insufficient to fully describe cardiac conduction.

Ephaptic coupling

As early as the 1960s, the hypothesis was put forth by Sperelakis and others that electrical excitation could be communicated between cardiac myocytes by means of an electric field or transient ion depletion/accumulation within the interstitial space (Sperelakis, 1969). These mechanisms, whereby propagation occurs without the electrotonic transfer of charge between myocytes via gap junctions, have been collectively termed 'ephaptic coupling'. Although initially proposed as a complete alternative to gap-junction coupling, interest in the ideas has been revived by modeling studies that have suggested the possibility of 'mixed mode' coupling wherein both mechanisms operate in tandem (Kucera et al., 2002; Lin & Keener, 2010; 2014; Mori et al., 2008). These models envision ephaptic coupling as occurring by means of transient depletion of sodium from the interstitial space during depolarization. Briefly, depolarization of an upstream myocyte results in its sodium channels withdrawing positive charge from the interstitium, thereby driving the local extracellular potential to more negative levels. Consequently, the potential difference across the downstream myocyte's membrane is elevated, activating its sodium channels and causing it to depolarize. Based on this picture of ephaptic coupling, these models identify two principal requirements for ephaptic coupling to occur: (a) close apposition (< 10 nm) of adjacent cell membranes, and (b) a high density of $\text{Na}_v1.5$ on the apposed membranes (Kucera et al., 2002; Lin & Keener, 2010, 2014; Mori et al., 2008; Copene & Keener, 2008; Sperelakis, 2002; 2003; Sperelakis et al., 1983; Sperelakis & McConnell, 2002). Based on these criteria, the observation of conduction slowing in response to increased interstitial volume (Veeraraghavan et al., 2012) would be expected if ephaptic coupling were involved in cardiac conduction. Further, the models also suggest that ephaptic coupling may sustain conduction when gap-junction coupling is reduced (Lin & Keener, 2013; 2014; Mori et al., 2008). This is consistent with the observation that increased interstitial volume unmasked conduction sensitivity to low levels of gap-junction uncoupling which on their own did not have a measurable impact (Veeraraghavan et al., 2012). While these results are certainly encouraging, the key to elucidate the role, if any, of ephaptic coupling in cardiac conduction will be the identification of the cardiac ephapse, a structure that can serve as the functional

unit of ephaptic coupling. Viewed in this light, recent insights into the structure of the ID raise some very interesting possibilities.

THE ID: THE MACHINERY OF CARDIAC CONDUCTION?

Given the close apposition between adjacent cell membranes required for ephaptic coupling, the ID is the logical place to start the search for the cardiac ephapse. Add to this the profusion of evidence that various ion channels, including the cardiac isoform of the $\text{Na}_v1.5$, are localized at the ID, (Kucera et al., 2002; Cerrone et al., 2013; Cohen, 1996; Hong et al., 2012; Lin et al., 2011; Maier et al., 2004; Malhotra et al., 2004; Milstein et al., 2012; Petitprez et al., 2011; Rasmussen et al., 2004) and it is highly likely that the ID is the most likely location for structures conducive to ephaptic coupling. The emerging picture is one of macromolecular complex(es) localized at the ID that contain not just ion channels but also adapter proteins (e.g. ankyrin-G; AnkG) and components previously identified with electrical (e.g. Cx43) and mechanical (e.g. plakophilin-2; PKP2) junctions (Cerrone et al., 2013; Geisler et al., 2010).

Sodium channels

Beyond just localizing to the ID, $\text{Na}_v1.5$ also interacts with various disk-localized proteins. It was demonstrated to co-immunoprecipitate with Cx43 (Malhotra et al., 2004) as well as to colocalize with it at the ID (Petitprez et al., 2011) in mouse hearts. On the structural side, it has been demonstrated that $\text{Na}_v1.5$ channels are preferentially localized at the ID compared with the lateral membrane of cardiac myocytes and are trafficked to these membrane sub-domains via distinct pathways (Petitprez et al., 2011). Functionally, this translates into elevated sodium current density at the ID compared with the lateral membrane (Lin et al., 2011) and *in silico* results have gone as far as suggesting that disk-localized sodium channels may be primarily responsible for depolarization while those on the lateral membrane help maintain the stability of conduction (Tsumoto et al., 2011).

Important work by Delmar and colleagues in recent years has shed light on the degree to which $\text{Na}_v1.5$ channels are integrated into the macromolecular complex at the ID. They have demonstrated that localization of mechanical junction proteins, mainly PKP2 (a desmosomal protein), precedes recruitment of Cx43 gap junctions and AnkG (a sub-membrane adapter protein involved in localizing $\text{Na}_v1.5$ in the membrane) to sites of cell-to-cell contact in neonatal rat ventricular myocytes (Geisler et al., 2010). Functionally, they have demonstrated that both regulation of the sodium current PKP2 and AnkG and linked mutations in both proteins to Brugada syndrome, an inherited arrhythmia syndrome resulting from loss of sodium current density (Cerrone et al., 2013; Hashemi et al., 2009). Additionally, they have reported reduced membrane levels of $\text{Na}_v1.5$, particularly at sites devoid of Cx43, in conditional Cx43 knockout mice (Jansen et al., 2012) and provided data suggesting that Cx43 may be involved in recruiting $\text{Na}_v1.5$ channels into the membrane (Agullo-Pascual et al., 2013; Delmar, 2012). Their observation of reduced $\text{Na}_v1.5$ membrane levels without accompanying loss of gap-junction coupling in mice with the last five C-terminal amino acids of Cx43 deleted lends further credence to this hypothesis (Lübke et al., 2013).

A key next step toward definitively the cardiac ephapse will be to locate microdomains within the ID that are rich in sodium channels and possess the requisite close apposition of membranes. In this context, a strong candidate that emerges is the perinexus—a membrane region located in the periphery of gap junctions where undocked connexon hemichannels localize (Rhett & Gourdie, 2012; Rhett et al., 2011a). Importantly, experiments using proximity ligation assays revealed fluorescent signal corresponding to the Cx43-Nav1.5 interaction localized at the perinexus (Rhett et al., 2012; Rhett et al., 2011b). Furthermore, the membranes of adjacent myocytes are very closely apposed at the perinexus by virtue of its location adjacent to the gap junction (Rhett et al., 2013). Likewise, work from the Delmar group has suggested the possibility of Nav1.5 channels localized near mechanical junctions, specifically desmosomes, (Cerrone et al., 2013; Noorman et al., 2013) which could potentially constitute yet another microdomain conducive to ephaptic coupling. Additionally, these developments suggest novel scaffolding roles for proteins such as Cx43 which have been predominantly associated with their junctional functions.

Overall, perijunctional regions within the ID possess the necessary close apposition of adjacent cell membranes for ephaptic coupling and the localization of Nav1.5 to these regions is a strong, albeit suggestive, clue. Research into ephaptic coupling in the heart should focus on these microdomains as the most logical candidate structures to function as the cardiac ephapse. And in a somewhat ironic turn of events, it might very well turn out that ephaptic coupling, which was initially viewed as an alternative to gap-junction coupling, might not only occur in tandem with it, but also be rooted at the edge of the gap junctions. In this light, even as we rethink the mechanisms of cardiac conduction, so too must we now rethink the roles of electrical and mechanical junction proteins: rather than merely being the site of intercellular electrical coupling, the ID might represent the active machinery of cardiac conduction.

Acknowledgments

This work was supported in part by grants from the National Institutes of Health (R01 HL102298-01A1 to SP, R01 HL56728-10A2 to RGG, R01-1DE019355-01 RGG subcontract), and American Heart Association Post-Doctoral Fellowship to RV. RGG holds stock in FirstString Research.

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