



Review

Old cogs, new tricks: A scaffolding role for connexin43 and a junctional role for sodium channels?



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ABSTRACT

Cardiac conduction is the process by which electrical excitation is communicated from cell to cell within the heart, triggering synchronous contraction of the myocardium. The role of conduction defects in precipitating life-threatening arrhythmias in various disease states has spurred scientific interest in the phenomenon. While the understanding of conduction has evolved greatly over the last century, the process has largely been thought to occur via movement of charge between cells via gap junctions. However, it has long been hypothesized that electrical coupling between cardiac myocytes could also occur ephaptically, without direct transfer of ions between cells. This review will focus on recent insights into cardiac myocyte intercalated disk ultrastructure and their implications for conduction research, particularly the ephaptic coupling hypothesis.

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Cardiac conduction is the process by which electrical excitation is communicated from cell to cell within the heart, triggering the synchronous contraction of the myocardium. Since being first demonstrated by Engelmann in 1874, [20] conduction has been the subject of intense scientific inquiry. Interest in the phenomenon stems mainly from the link between aberrant conduction and potentially lethal arrhythmias in a variety of pathologies.

1. Historical background

The current understanding of conduction is based largely upon the core conductor model [45]. The roots of this theoretical paradigm can be traced back to the application of continuous cable theory to cardiac conduction by Silvio Weidmann in the 1950s [75]. Subsequent experimental results, while numerous, have largely fit into the framework of this model, which envisions conduction as having two functional components: Membrane excitability and intercellular coupling. Membrane excitability, or the ability of an excitable membrane to depolarize in response to a given stimulus, is thought to be the province of membrane ion channels, particularly voltage-gated sodium channels. Intercellular coupling

is seen as occurring via the passive, electrotonic flow of positive charge between cells via low resistance pathways afforded by gap junction (Gj) channels. However, emerging experimental evidence suggests that this view, while perhaps tidy, may not offer a complete and accurate description of cardiac conduction. For a detailed discussion of the electrotonic model of cardiac conduction, the reader is referred to the previous reviews by Spach et al. [58] and Kleber & Rudy [29].

The challenge to the electrotonic model of cardiac conduction comes in the form of ephaptic coupling, a process by which electrical excitation is communicated between cells via an extracellular electric field or ion accumulation/depletion without involvement of Gjs [61,65]. This mechanism, known to occur in other excitable tissues such as the brain, the retina and the uterine myometrium, [28,74,76] has long been hypothesized to play a role in cardiac conduction by Nicholas Sperelakis and others [12,33,40,42,63,64]. However, the lack of direct experimental evidence and a well-defined functional unit, i.e., an ephapse, has meant that the investigation of ephaptic coupling has remained almost exclusively the province of mathematical models. In this article, we will focus emerging evidence for ephaptic coupling in the heart and their theoretical implications – in particular, new functions for Gjs and voltage-gated sodium channels, blurring the boundary between excitability and intercellular coupling.

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2. Intercellular coupling: gap junctions and beyond

Over the last century, our understanding of cardiac intercellular coupling has gone through a series of revisions. In the early days of conduction research, the cytoplasm of cardiac myocytes were thought to be contiguous, thus accounting for electrical coupling. However, with the identification of high resistance membrane bounding each myocyte, [56] it was postulated that there had to exist low resistance pathways coupling neighboring myocytes [13]. Using electron microscopy to study the intercalated disk at high resolution, Dewey, Sjostrand and Andersson suggested that it may constitute a connecting surface between myocytes [55]. Subsequently, in the early 1960's, using electron microscopy, Lloyd Barr and colleagues identified 'fused membrane' structures connecting adjacent myocytes, which they dubbed *the nexus* [18]. Around the same time, Van der Kloot and Dane proposed the intercalated disk as the likely site of low resistance electrical contact between myocytes [72]; shortly thereafter, Barr, Dewey and Berger provided direct evidence of the nexus's involvement in conduction [5]. In 1967, Revel and Karnovsky demonstrated the nexus to be membranes separated by a gap rather than fused and coined the term 'gap junctions' [46]. The resistance of GJ was initially considered to be low enough to render coupled myocytes electrically continuous, thus conferring a syncytial nature upon the myocardium. However, experimental studies of action potential propagation at high temporal resolution revealed GJ resistance to be high enough to render cardiac conduction discontinuous at the cellular level [60].

Cardiac GJs have long been recognized to undergo remodeling in developmental [3,23] and disease scenarios [36,43,57]. In this regard, one key question has been the precise relationship between the degree of GJ uncoupling and the resulting level of conduction slowing. While conduction slowing in response to pharmacological uncoupling has been well characterized, [4,7,15,17,26,30,52] the electrophysiological impact of pathophysiological GJ remodeling is less clear [2,8]. Experiments in transgenic mice with 50% reduced expression of connexin43 (Cx43), the principal ventricular GJ protein, have yielded mixed results: Some studies reported slower conduction compared to wild-type (WT) littermates [19,24] while others found no difference [6,41,67,68,70,71]. Even more perplexingly, conduction, albeit slowed and susceptible to failure, still occurs in mice with a cardiac-specific conditional knockout of Cx43 resulting in a severe (>80%) loss of Cx43 [14].

All these findings point back to a question first posed by Sperelakis during the 1960's [62]: Can ephaptic coupling sustain cardiac conduction in the absence of GJs? While initially viewed as an alternative to GJ coupling, more recent *in silico* studies have suggested the possibility of so-called *mixed-mode* coupling involving both mechanisms [31–33,40]. These models envision intercellular coupling as occurring as follows: A depolarized myocyte withdraws sodium ions from the restricted junctional cleft via its intercalated disk-localized $\text{Na}_v1.5$ channels (Fig. 1A). The resulting depletion of positive charge from the junctional cleft would render the local extracellular electrical potential more negative. Consequently, the transmembrane potential across the apposed membrane of the neighboring myocyte becomes more positive, causing the activation of $\text{Na}_v1.5$ channels (Fig. 1B). Thus electrical activation is communicated from myocyte to myocyte without direct transfer of ions between them (Fig. 1C). Based on this view, the models almost unanimously predict that ephaptic coupling would require that:

- (a) the membranes of adjacent myocytes are closely apposed (<10 nm apart) and,

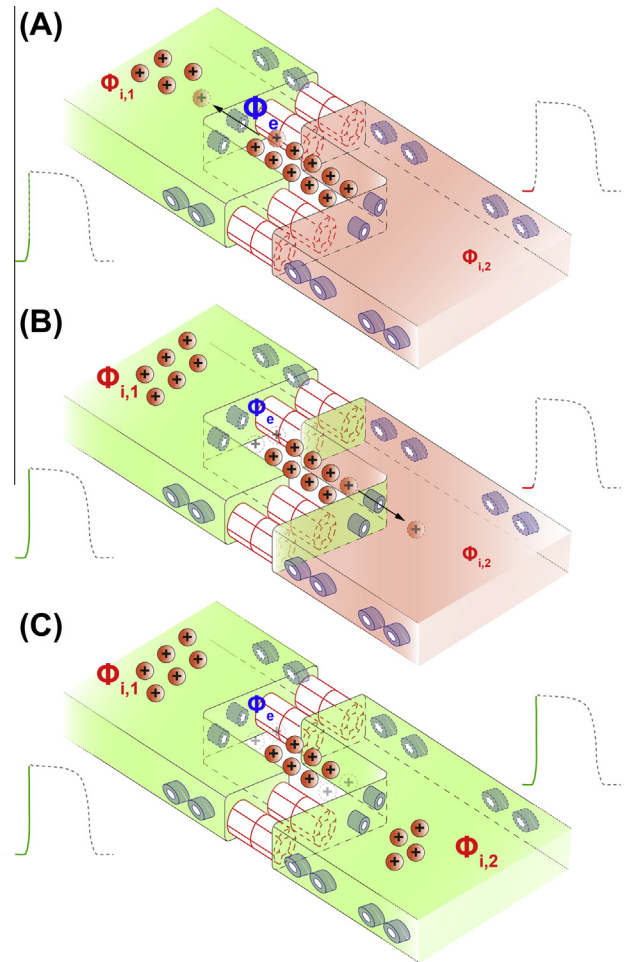


Fig. 1. Schematic cartoon illustrating the mechanism of ephaptic coupling. (A) Sodium channels (shown in blue) on the depolarized myocyte's membrane activate, withdrawing positively charged sodium ions (Na^+) from the restricted extracellular cleft at the intercalated disk. This raises the intracellular potential ($\Phi_{i,1}$) of the first myocyte. (B) Concomitantly, the depletion of positive charge from the restricted extracellular cleft lowers the local extracellular potential (Φ_e). There is a resultant increase in the transmembrane potential across the membrane of the second myocyte which is defined as the difference between its intracellular potential ($\Phi_{i,2}$) and the extracellular potential (Φ_e). In turn sodium channels located at or near the intercalated disk of the second myocyte activate. (C) Entry of sodium ions into the second myocyte via its sodium channels further depolarize it, triggering an action potential. Thus activation is communicated 'ephaptically' from cell-to-cell without the direct transfer of ions between them.

- (b) the closely apposed membranes are rich in cardiac sodium channels ($\text{Na}_v1.5$) [12,31–33,40,63,64,66].

3. Ion channels at the intercalated disk: functional implications

Recent insights into the ultrastructural organization of ion channels within cardiac myocytes have sparked interest in the ephaptic coupling hypothesis, particularly when interpreted in the context of the aforementioned model predictions. The first evidence that cardiac sodium channels are preferentially localized at the intercalated disks of cardiac myocytes came in 1996, when Dr. Sidney Cohen published immunofluorescence images of rat TTX-resistant sodium channels (rH1) [11]. Since then there has been mounting evidence for the intercalated disk localization of ion channels, long predicted by mathematical models as a requirement for ephaptic coupling [12,31,39,40,61,65,77]. Since then, other studies have recapitulated the preferential localization of

cardiac sodium channels ($\text{Na}_v1.5$) to the intercalated disk [31,37]. More importantly, Dr. Kucera and colleagues demonstrated in a 1D strand model of cardiac conduction that the high density of sodium channels at the intercalated disk could impact cardiac conduction in previously un-appreciated ways: Specifically, they concluded that G_j are still likely the principal mechanism of electrical transmission between cells, however, sodium channels at the intercalated disk could couple myocytes ephaptically, particularly when G_j coupling is compromised.

Since then a more detailed picture of proteins at the intercalated disk has emerged, revealing the existence of a macromolecular complex containing Cx43, cardiac sodium channels ($\text{Na}_v1.5$) and various cytoskeletal proteins. Cx43 and $\text{Na}_v1.5$ have been demonstrated to co-immunoprecipitate from mouse heart lysates [38] and to colocalize at the intercalated disk [44]. Dr. Mario Delmar and colleagues have provided evidence suggesting that Cx43 and $\text{Na}_v1.5$ participate in a macromolecular complex at the intercalated disk which includes the desmosomal protein plakophilin-2 (PKP2) as well as ankyrin-G, a sub-membrane adapter protein involved in localizing cardiac sodium channels ($\text{Na}_v1.5$) in the membrane. In primary cultures of neonatal rat ventricular myocytes, they found that Cx43 gap junctions and ankyrin-G (AnkG) are recruited to sites of cell-to-cell contact following the localization of mechanical adhesion proteins [22]. Subsequently, they demonstrated regulation of the sodium current by both PKP2 and AnkG [10,53,54] and mutations in both proteins have been associated with Brugada syndrome, an inherited arrhythmia syndrome characterized by decreased sodium current density [9,25]. Recently, Dr. Delmar and colleagues reported loss of $\text{Na}_v1.5$ from the membrane in conditional Cx43 knockout mice [27] and suggested that Cx43 play a role in the recruitment of $\text{Na}_v1.5$ channels into the intercalated disk membrane [1,16]. Further support for this hypothesis comes from their observation of decreased sodium and potassium current levels as well as loss of $\text{Na}_v1.5$ from the intercalated disk without any concomitant loss of G_j coupling in mice lacking the last 5 C-terminal amino acids of Cx43 [35]. In all, emerging evidence both structural [44] and functional [34] suggests the existence of two distinct pools of $\text{Na}_v1.5$ located at the intercalated disk and at the lateral membrane.

These observations resonate with computer models which suggest that intercalated disk localized sodium channels may be involved in ephaptic coupling [31–33], while those on the lateral membrane are important for maintaining the stability of conduction [69], particularly when G_j coupling is reduced. In all there is mounting support for the hypothesis that microdomains could exist within the intercalated disk that have the necessary density of sodium channels for ephaptic coupling to occur. Close apposition between cell membranes, the other criterion for ephaptic coupling predicted by models, could help identify specific structures that might function as a cardiac ephapse.

4. Ephaptic coupling: experimental traces

The functional observations suggesting a role for ephaptic coupling in the heart come from investigation of conduction dependence on interstitial volume. Under the electrotonic view of conduction, the interstitial space provides the return path for electrical current flowing between myocytes, thus completing the circuit. Based on this notion, conduction velocity should be directly proportional to interstitial volume, [45,59] and observations in cable-like papillary muscle have been consistent with this notion [21]. However, we recently demonstrated that increasing interstitial volume in a heart slows conduction and vice versa, i.e. an inverse relationship between conduction velocity and interstitial volume [73]: These observations are inconsistent with a purely

electrotonic view of cardiac conduction. Additionally, we found that levels of G_j uncoupling too small to alter conduction normally, significantly slowed conduction when the interstitial volume was increased. These findings are consistent with the hypothesis that increased interstitial volume impairs ephaptic coupling, thus slowing conduction and increasing its dependence on G_j coupling. These results underscore the importance of interstitial volume, i.e. the spacing between membranes of adjacent myocytes, as a determinant of conduction and offer further impetus for a critical reassessment of the role ephaptic coupling may play in cardiac conduction. Additionally, they dovetail with the predictions made by computer models of ephaptic coupling [31,32,40]. However, as previously stated, any attempt to directly assess whether ephaptic coupling plays a role in cardiac conduction must first contend with the question of its structural underpinnings. In other words, a functional unit of ephaptic coupling, an ephapse, will need to be identified.

5. Ultrastructural breadcrumbs leading to the ephapse?

Taking together the aforementioned structural and functional insights in the context of the *in silico* predictions, it could be hypothesized that the cardiac ephapse is likely to be a microdomain within the intercalated disk with a high density of cardiac sodium channels ($\text{Na}_v1.5$) and close apposition between the membranes of adjacent myocytes.

One structure that emerges as a promising candidate for the ephapse is the perinexus – a specialized membrane microdomain surrounding G_j plaques and rich in undocked connexon hemichannels [47,48]. While previous studies identified the interaction between Cx43 and $\text{Na}_v1.5$ at the intercalated disk [35,37,38,53], proximity ligation assays (PLA – e.g., Duolink) of protein–protein association enabled imaging of Cx43– $\text{Na}_v1.5$ interaction at the perinexus [49,50]. By virtue of its location at the periphery of G_j plaques, the perinexus features close apposition between the membranes of adjacent myocytes [51]. This feature, together with the focal concentrations of sodium channels generated by a scaffold that includes Cx43 hemichannels, indicates that the perinexus potentially meets both criteria identified by mathematical models to function as an ephapse between cardiac myocytes.

Further studies utilizing emerging modalities such as super-resolution microscopy and the PLA interaction assay, combined with the high resolution of electron microscopy will be critical to fleshing out the structure of the macromolecular complex located at the intercalated disk. As the constituents of this complex are identified, functional experiments in intact myocardium will be needed in order to elucidate their roles in forming and maintaining the machinery of cardiac conduction. And, identifying the ephapse will only be the first step in formulating a new theory of cardiac conduction. Fuller appreciation of the role of ephaptic coupling, will require a two-pronged approach: (a) Further experiments to investigate the relationship between the molecular ultrastructure of the intercalated disk and the electrophysiology of the heart; and (b) Structurally detailed, multi-dimensional mathematical models which incorporate ephaptic coupling to probe the mechanisms underlying the new experimental observations.

6. Conduction: a new multi-factorial understanding

Continuing the trend of the last 130 years, our understanding of cardiac conduction appears to be on the verge of yet another revision – and perhaps a shift in paradigm. The primary driver of this change is the new picture that we are obtaining of the intercalated disk – the prime locus of the machinery of cardiac conduction. With respect to its role in cellular contact and communication,

the intercalated disk is an intricate, dynamically regulated machine rather than a simple, naïve structure. Continuing a trend that began a decade or so ago, we are beginning to see connexins not just as channels, but as multi-functioned constituents of macromolecular complexes, heralding a new chapter in the biology of these molecules. As we understand myocyte structure at finer and finer resolution, so are we also beginning to appreciate the importance of biophysical phenomena that occur at the scale of nanometers. The erosion of existing conceptual boundaries, such as that between tissue excitability and intercellular coupling, is leading to a new, multi-factorial view of conduction where the same molecules appear to play several roles and multiple mechanisms work in tandem to achieve a single function. This deeper understanding could help explain the processes underlying pathological conduction defects and open up novel avenues for therapy.

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