Conduction in Normal and Diseased Myocardium

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What You Will Learn in This Chapter

This chapter discusses the passive component of conduction that occurs between electrically coupled cells. In addition, the necessary conditions for wavefront propagation and thus normal cardiac function are introduced. Finally, this chapter will explore how intercellular coupling can be altered in pathology, and the effects this has on the electrical function of the heart.

Learning Objectives

- Understand existing theories of cardiac conduction at both a gross and molecular level
- Be able to discuss gaps in current knowledge, including the variable functional effects of phosphorylation status at serine residues
- Be able to give two examples of neoteric therapies specific to cardiac conduction

7.1 Cardiac Conduction and Gap Junctions

Cardiac conduction refers to the propagation of electrical activity through the heart and can be separated into active and passive components. The active component relies on membrane-bound events that are responsible for the cell's response to excitation, previously discussed in ▶ Chap. 5. This section will discuss the passive component of conduction, occurring between electrically coupled cells.

Cardiac myocytes are electrically coupled through gap junctions, which provide a conduit for charged particles and small molecules of up to 1000 Daltons in size. Gap junctions are predominantly located at the intercalated discs, where they form clusters known as *plaques* [1].

Individual gap junctions consist of many gap junctional channels, which in turn are formed by the union of two hemi-channels contained on the surface of two cells in contact with one another. The hemi-channel, or *connexon*, is in turn formed by six *connexin* proteins.

Many different connexin proteins have been identified in the human heart, each with different molecular weights and functional properties. Their nomenclature is based on their molecular weight. The most prevalent types are connexin40 (Cx40), Cx43, and Cx45. The distribution of these connexins in the mammalian heart is shown in ◘ Fig. 7.1. Broadly, gap junctions are formed by Cx43 in ventricular myocytes and Cx40 and Cx43 in atrial myocytes, with Cx45 of particular importance in the specialised conduction tissues. Due to the distribution of connexins varying between species, experimental findings in animal models may not be directly transferable to humans [2, 3].

7.1.1 Safety of Conduction

The *safety factor* (SF) for conduction describes the likelihood of successful action potential propagation across the myocardium and is determined by the relationship between two variables. The '*source*' refers to excited cells which provide charge to depolarise neighbouring unexcited cells, acting as an electrical 'sink' or load. For propagation to succeed, the source must overcome the sink, described by a SF of 1 or more. When the source provides insufficient charge to depolarise the sink, SF falls to below 1 and conduction block occurs [4].

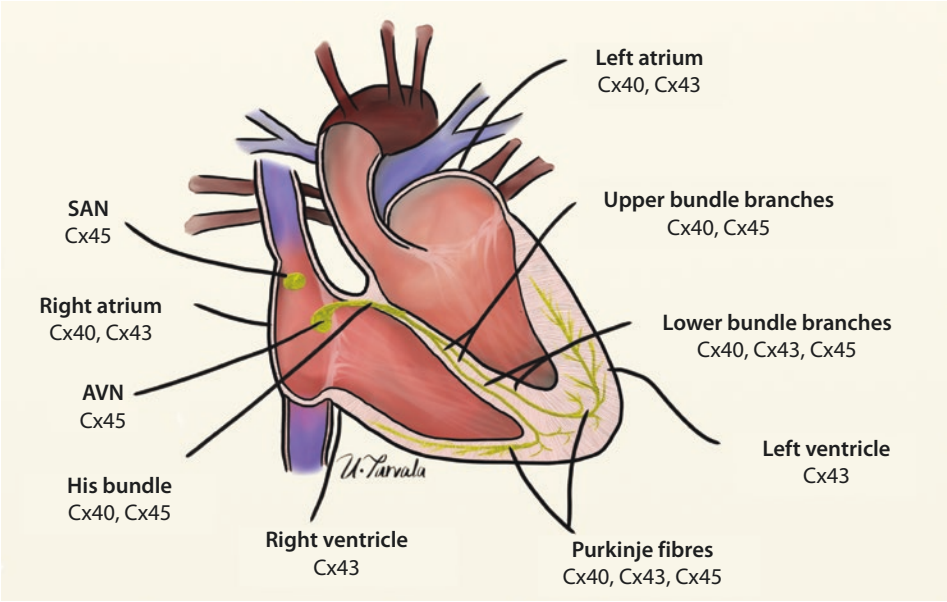


Fig. 7.1 Diagram of the pattern of expression for the three principle connexins in the mammalian heart

Table 7.1 Table showing safety factor for conduction and conduction velocity at different levels of inter-cellular coupling

Tissue	Strong intercellular coupling	Moderate intercellular coupling	Weak intercellular coupling
Safety factor	Moderate	High	Low
Conduction velocity	Rapid	Slow	Very slow

The importance of SF can be illustrated by modelling the propagation of an afterdepolarisation originating from a cluster of contiguous myocytes. In well-coupled tissue, this cluster of myocytes is electrically connected to many unexcited cells, which act as a large electrical sink. This means that the SF is low, and afterdepolarisations rarely propagate into the surrounding myocardium. In poorly coupled tissue, the sink is comparatively smaller and afterdepolarisations are therefore much more likely to spread, resulting in a premature ventricular complex [5].

Intercellular coupling is also an important determinant of conduction velocity, evidenced by the reduced conduction velocity observed in human and human and guinea-pig myocardium that has previously undergone the pharmacological uncoupling of gap junctions using carbenoxolone [4, 6] (Table 7.1).

7.1.2 Gap Junction Function in Cardiovascular Disease

A variety of cardiac diseases are known to alter the gap junction function through a multitude of different mechanisms. Reduced expression of Cx43 has been observed in animal models of heart failure, associated with both reduced conduction velocity and increased transmural dispersion of repolarisation. These are both pro-arrhythmic [7].

Changes to the phosphorylation status of the carboxy terminus of Cx43 are also known to modulate gap junction function. This has been explored using Cx43 knock-in mice with either a phosphomimetic glutamic acids, or with non-phosphorylatable alanines contained within the carboxy terminus. The former is resistant to gap junction remodelling following acute (global ischaemia) and chronic (trans-aortic constriction) pathological stimuli, in addition to displaying reduced susceptibility to induced ventricular arrhythmias. In contrast, the latter exhibit aberrant Cx43 expression at baseline and a greater susceptibility to ventricular arrhythmia compared to wild type controls [8].

The arrhythmogenic effects of Cx43 dephosphorylation are of particular importance during myocardial ischaemia. In control hearts, almost all of the Cx43 present at intercellular junctions is phosphorylated. Experimental models have demonstrated that ischaemia does not alter the total amount of Cx43, but reduces phosphorylated Cx43 in a time-dependent manner [9].

Perturbations of connexin trafficking can also reduce intercellular coupling. Microtubules have a well-characterised role in transporting Cx43 hemi-channels to the plasma membrane where they are able to form functional gap junctions. Ischaemic human hearts have displaced microtubules-associated proteins. This limits the delivery of Cx43 to its canonical position at the intercalated disc and consequently reduces intercellular coupling [10].

Gap junction localisation may also be affected by alterations to accessory proteins. One of the most well-explored examples is zonula occludens (ZO)-1, which interacts with Cx43. Interfering with the Cx43–ZO-1 interaction leads to increased gap junction plaque size, suggesting that ZO-1 regulates the accretion of Cx43-containing gap junctions [1]. Whilst it is clear that ZO-1 possesses the ability to modulate gap junction localisation, its role in disease is less clear. Failing human hearts display a 95% reduction in ZO-1 expression, but this is accompanied by a paradoxical reduction in Cx43 staining at intercalated discs [11]. Clearly, more work is required to understand the effects of ZO-1 in health and disease.

7.1.3 Proarrhythmic Effects of Altered Gap Junction Function

Alterations to cellular coupling are closely associated with the formation of re-entrant arrhythmias, but it is important to note that re-entry may also result from numerous other functional and structural modifications of the myocardium. This form of conduction occurs when a propagating wavefront encounters an obstacle and circulates around the area to re-excite tissue at the site of origin. This rotating wavefront may proceed through several cycles, repeatedly exciting the surrounding myocardium. This is in stark contrast to normal cardiac conduction, in which a wavefront originates from the cardiac pacemaker and activation and recovery of the myocardium are completed before the arrival of the next stimulus [12].

Re-entry relies on both the conduction velocity (Θ) and refractory period (t_r). The product of these two variables defines the wavelength of excitation (λ; $\lambda = \Theta t_r$). Re-entry can occur when the length of the re-entrant circuit is greater than or equal to the wavelength of excitation.

Under these conditions, a so-called *excitable gap* is formed: excitable tissue that lies between the head of the circulating wavefront and the repolarising tail of the preceding wave. Impaired intercellular coupling reduces conduction velocity and thus λ. This renders the myocardium more susceptible to arrhythmia by reducing the minimum path length necessary for re-entry [4].

Re-entrant arrhythmias include atrial fibrillation, atrial flutter, ventricular tachycardia and ventricular fibrillation. Peters et al. were among the first to explicitly correlate gap junctional changes with re-entrant circuits [13]. Myocardial infarction was generated in six mongrel dogs by surgical ligation of the left anterior descending artery (LAD). Four days later, an electrode array was used to produce activation maps of the myocardium during induced ventricular tachycardia (VT). These maps showed two connected re-entrant circuits; one circulating clockwise and the other counter-clockwise. Both circuits travel through a central passage termed the central common pathway, giving rise to a characteristic figure-of-eight shape.

In the same study, sections of myocardium were also immunolabelled for Cx43. Myocytes in the sub-epicardial tissue overlying the infarcted region (the so-called *epicardial border zone*, *EBZ*) displayed Cx43 abnormally located on the lateral surface of cells. In some regions, cells with altered gap junction distribution were present throughout the entire EBZ, extending all the way to the epicardial surface. Crucially, these regions of full-thickness gap junction disarray were shown to correlate with the location of the central common pathway of circuits responsible for VT [13].

7.1.4 Functional Effects of Gap Junction Lateralisation

Gap junction lateralisation has been noted in numerous pathological conditions, but its functional effects remain unclear. In healthy myocardium, the predominant localisation of gap junctions at intercalated discs helps to establish anisotropic conduction. This refers to conduction that occurs at higher velocity in the longitudinal than transverse direction. Lateralisation would be expected to increase gap junction current travelling in a transverse direction, thus reducing anisotropy. This hypothesis has been explored in the healing canine infarct model described above. Surprisingly, there is normal transverse conductance between cells in the common central pathway with lateralised gap junctions [14].

Other groups have even found a paradoxical reduction in side-to-side coupling in these myocytes, raising the possibility that lateralised gap junctions are non-functional [15]. They may merely be a marker of disease, rather than an active participant in altered wavefront propagation. Interestingly, gap junction conductance in the longitudinal direction was normal in both of these studies, but conduction slowing through the common central pathway was present nonetheless. This has been attributed to modulation of sarcolemmal ion currents such as I_{Na}, and illustrates the importance of considering both the active and passive components of cardiac conduction [14].

7.2 What We Don't Know

7.2.1 Functional Effects of Phosphorylation Status at Serine Residues

As previously described, myocardial ischaemia is known to result in the dephosphorylation of Cx43. Whilst this statement is generally true, it fails to acknowledge the role of specific serine residues of Cx43. Some of these respond to ischaemia in the manner expected with, for example, an eight-fold reduction in the presence of Cx43 phosphorylated at serines 325/328/330 in ischaemic myocardium. The functional importance of this can be demonstrated by transfecting $Cx43^{-/-}$ mice with a mutated form of Cx43 in which these three serine residues cannot be phosphorylated. In these cells, the event frequency for fully open channels was markedly lower than in wild type controls. Moreover, they also displayed impaired transfer of Lucifer Yellow dye between cells, which is a surrogate marker of gap junction conductance [16].

In contrast, some serine residues appear to be phosphorylated in response to ischaemia. Serine 368 phosphorylation is increased following the imposition of no-flow ischaemia. Cx43 phosphorylated in this manner remains predominantly located at the intercalated disk, despite extensive lateralisation of total Cx43.

However, the functional implications of this modification remain unclear. Serine 368 phosphorylation might reduce total channel conductance and ion permeability [17]. Further complexity is added by considering the regulatory role of Cx43 residues. Phosphorylation of serine 365 is ubiquitous in homeostatic cells, however is lost following ischaemia. This is associated with more efficient phosphorylation of serine 368. It is therefore possible that phosphorylation of Cx43 at serine 365 protects cells against the effects of ischemia by limiting serine 368 phosphorylation [18].

All of these studies illustrate the complex and sometimes contrasting alterations to the phosphorylation state of Cx43 under conditions of ischaemia. The functional implications of serine residue phosphorylation in response to ischaemia are yet to be fully characterised.

7.2.2 Non-Gap Junctional Transmission of Electrical Impulses

Some of the most curious findings in the field of cardiac conduction have been made in connexin knock-out mice. It is evident that cardiac-restricted inactivation of Cx43 almost abolishes side-to-side and end-to-end gap junctional coupling between cell pairs. From these findings, it might be assumed that wavefront propagation on the whole-heart level would also be abolished. In fact, conduction velocity is reduced by just 50% in both the longitudinal and transverse directions [19].

This might be explained by the ephaptic coupling hypothesis. Conduction through this mechanism relies on close spatial apposition of adjacent cell membranes and on a high density of sarcolemmal $Na_v1.5$ channels. Under these conditions, myocyte depolarisation is hypothesised to draw Na^+ ions from the cleft between apposed cells, with the removal of positive ions making the voltage of the cleft more negative. The potential difference between the extracellular space and the neighbouring myocyte is therefore more positive. If a sufficiently positive potential differences is reached, $Na_v1.5$ channels in the neighbouring cell would open, allowing excitation to propagate from one cell to another in a gap junction-independent manner [2].

In reality, conduction is likely to occur through both gap junctional and ephaptic mechanisms, in so-called '*mixed mode*' coupling, supported by computation tissue models showing that enhanced ephaptic coupling reduces the gap junctional coupling necessary for successful conduction [20]. The mechanisms underlying cell-to-cell electrical propagation, and their relative importance, are yet to be fully elucidated.

7.3 Where We're Heading

7.3.1 Pharmacological Enhancers of Gap Junction Function

Rotigaptide (ZP-123) is an under-investigation clinical compound that has been suggested as a novel treatment for cardiac arrhythmias due to its enhancement of gap junctional coupling.

Initial experiments on this drug focused on its potential influence on the acute electrophysiological response to ischaemia, with myocardial infarction induced by surgical ligation of the left anterior descending (LAD) artery in mongrel dogs. Infusion of rotigaptide significantly reduced the incidence of induced VT in the 1–4 hours post-infarction [21]. Whilst this study demonstrated the potentially beneficial effects of rotigaptide in the context of infarction, it did not explore whether this benefit could be extended beyond the course of a few hours.

More recently, attention has turned towards the potential role of rotigaptide in influencing the pathophysiological processes that underlie infarction, meaning it may confer protection against arrhythmia that extends beyond the duration of treatment. This has been explored in rats given a loading dose of rotigaptide immediately before ligation of the LAD and for the subsequent 7 days. Rotigaptide-treated hearts had reduced arrhythmia susceptibility at 4 weeks post-infarction that could not be explained by a reduction in Cx43 lateralisation or scar size.

The anti-arrhythmic effect is most likely to result from a less heterogenous pattern of fibrosis at the infarct border zone (IBZ). Heterogeneity of scarring in this region is known to provide a substrate for re-entry, as the interdigitation of myocardium and fibrosis produces a long and tortuous path of conduction. There was also a concurrent increase in conduction velocity across the IBZ, which would reduce the wavelength of excitation and thus the occurrence of re-entry.

Gap junction enhancement is hypothesised to be the mechanism responsible for these observations. Closure of gap junctions during ischaemia prevents the cell-to-cell transfer of mediators of cell death and survival. This isolates cells within a cluster, meaning there is likely to be a greater spread of cell survival and death. Enhancement of gap junction conductance with rotigaptide would allow these mediators to be shared between surrounding cells, producing clusters of cells with a shared fate [22]. More work is required to establish whether the beneficial effects of rotigaptide in animal models can be translated to humans.

7.3.2 Modulation of Accessory Proteins

Modulation of accessory proteins is another proposed mechanism to therapeutically augment gap junction function. αCT1 is a peptide memetic of carboxyl terminus of Cx43 that has been shown to reduce the interaction between Cx43 and the accessory protein ZO-1. As discussed previously, this has been shown to increase gap junction plaque size in vitro [1].

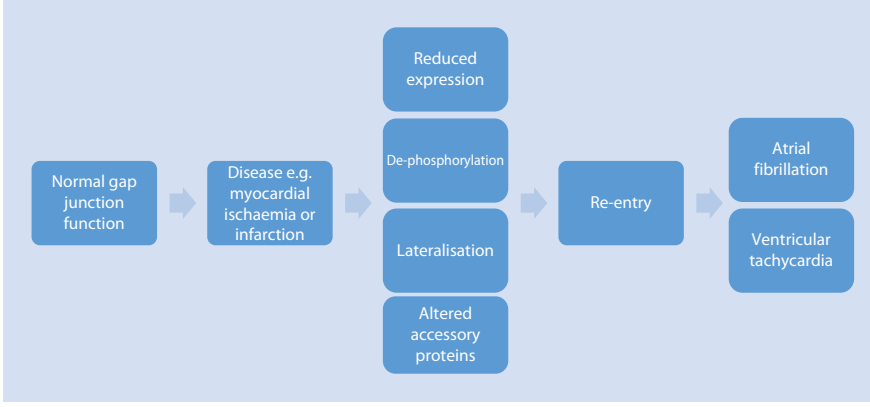
Its effects in vivo have been explored using mice exposed to cryo-injury of the left ventricle. Whilst this model has little clinical relevance, it is proposed to generate a more reproducible injury than coronary artery ligation. Injuries were immediately covered with a patch containing αCT1 or vehicle solution. At 7–9 days post-injury, αCT1-treated hearts showed reduced susceptibility to induced arrhythmia, with an increased ventricular depolarisation rate. This was associated with a significant reduction in Cx43 lateralisation in the injury border zone of αCT1-treated hearts. Surprisingly, this may be due to altered Cx43 phosphorylation rather than modulation of the effects of ZO-1. Compared to controls, αCT1-treated hearts had significantly higher levels of Cx43 phosphorylated at serine 368 [23].

This form of Cx43 has been shown as preferentially localised to the intercalated disc following ischaemia [17] and might improve intercellular coupling to thus reduce the propensity for arrhythmias. This illustrates the anti-arrhythmic effects of this mechanism of gap junction modulation, but their clinical relevance is limited by the selected model and route of drug delivery.

7

Take-Home Message

- Normal cardiac conduction synchronises the activity of cardiomyocytes, giving rise to coordinated and efficient mechanical contraction.
- Propagation of electrical activity across the myocardium is due in part to gap junctions, which facilitate the transfer of small molecules between neighbouring myocytes.
- Gap junction changes in disease interfere with wavefront propagation, increasing the incidence of re-entry.
- Novel therapies aim to modulate gap junction function following myocardial injury, reducing susceptibility to arrhythmia.



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