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Can triggered arrhythmias arise from propagation of Ca^{2+} waves between cardiac cells?

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1. ABSTRACT

Intracellular Ca^{2+} overload can induce regenerative Ca^{2+} waves that activate inward current in cardiac myocytes, allowing the cell membrane to achieve threshold. The result is a triggered extrasystole that can, under the right conditions, lead to sustained triggered arrhythmias. In this review, we consider the issue of whether or not Ca^{2+} waves can travel between neighboring myocytes and if this intercellular Ca^{2+} diffusion can involve enough cells over a short enough period of time to actually induce triggered activity in the heart. This review is not intended to serve as an exhaustive review of the literature summarizing Ca^{2+} flux through cardiac gap junctions or of how Ca^{2+} waves move from cell to cell. Rather, it summarizes many of the pertinent experimental studies and considers their results in the theoretical context of whether or not the intercellular propagation of Ca^{2+} overload can contribute to triggered beats and arrhythmias in the intact heart.

2. INTRODUCTION

Abnormal intracellular Ca^{2+} cycling is responsible for triggered arrhythmias, particularly in the setting of intracellular Ca^{2+} overload that results from ischemia, cell damage, pharmacological or neurohormonal alterations in intracellular Ca^{2+} concentration (e.g. digitalis toxicity and β-adrenergic stimulation), genetic mutations in Ca^{2+} cycling proteins (e.g. mutations of the cardiac ryanodine receptor, RyR2, or calsequestrin in catecholaminergic polymorphic ventricular tachycardia), and other interventions that alter Ca^{2+} homeostasis. It is well known how altered Ca^{2+} cycling in the form of spontaneous Ca^{2+} waves can induce cellular depolarization and how this cellular depolarization can achieve the voltage threshold for activation of inward Na^{+} current, producing an extrasystole. However, it is essential for this aberrant excitation to spread among a sufficient number of cells across a sufficiently large mass of ventricular tissue (the liminal mass) in order to induce a triggered beat that conducts throughout the myocardium.

One of the ways that has been proposed to spread enough depolarizing current to achieve threshold in intact heart is that Ca^{2+} waves might spread from a focal region of intracellular Ca^{2+} overload to neighboring myocytes through gap junctions, thus spreading depolarization by involving enough adjoining tissue to bring it to threshold to produce a conducted beat. It is important to note, however, that the spread of Ca^{2+} between myocytes is carefully regulated by the myocyte through regulation of gap junctions which are responsible for physiologically essential functions of maintaining small substrate, metabolic and ionic balance among cells. In addition, regulation of intercellular communication also insures that pathophysiological processes (including Ca^{2+} overload) do not spread easily between myocytes, thus limiting the spread of tissue damage.

The purpose of this work is to review what is known about the diffusion of Ca^{2+} between cardiac myocytes and to consider how Ca^{2+} spread from cell to cell might be involved in the generation of triggered arrhythmias.

3. INTRACELLULAR Ca^{2+} CYCLING AND ARRHYTHMIAS

Excitation-contraction coupling (ECC) is a process that allows the heart to pump blood efficiently throughout the body (1,2). ECC begins with activation of the ventricular action potential, which depolarizes the cell and triggers calcium-induced calcium release (CICR), resulting in intracellular Ca^{2+} cycling. Once the action potential has spread throughout the cell, Ca^{2+} enters through L-type Ca^{2+} channels found primarily on the plasma membrane in the t-tubules that spread activation to the cell core. This entering Ca^{2+} binds to and activates ryanodine receptors (RyR) on the sarcoplasmic reticulum (SR), inducing the release of more Ca^{2+} from this Ca^{2+} storage organelle into the cytosol, in a local event known as a Ca^{2+} spark. The summation of thousands of Ca^{2+} sparks throughout the cell produces a homogeneous rise in Ca^{2+} throughout the cell, resulting in the formation of Ca^{2+} transients. This rise in cytosolic Ca^{2+} causes the interaction between actin and myosin filaments, resulting in cardiac contraction. Once contraction is completed, most Ca^{2+} is pumped back into the SR while some exits the cell via the sodium-calcium exchanger (NCX), thus restoring Ca^{2+} gradients in time for the next cardiac cycle.

Under abnormal conditions, such as during myocardial damage, excessive phosphorylation, excessive Ca^{2+} influx and Ca^{2+} mismanagement of Ca^{2+} release by genetically or pharmacologically altered protein function, a state known as Ca^{2+} overload is induced. Under these conditions, the SR becomes overloaded with Ca^{2+} and cannot sequester enough to restore the normal Ca^{2+} balances. Because of the high SR Ca^{2+} content, RyRs open spontaneously (in the absence of L-type Ca^{2+} influx and CICR) causing Ca^{2+} release that spreads from release unit to release unit, in a regenerative manner as a propagating Ca^{2+} wave (2). As Ca^{2+} overload progresses, the high Ca^{2+} sensitivity of RyRs and SR Ca^{2+} overload cause the continuous generation of abnormal Ca^{2+} waves (2,3). Most importantly, Ca^{2+} waves activate the sarcolemmal Na+-Ca^{2+} exchanger (NCX), an equilibrium exchanger that is also electrogenic. The result is that removal of the excess Ca^{2+} released spontaneously by the SR during a wave also produces inward depolarizing current which can be of sufficient magnitude and timing to cause a cell depolarization above the voltage threshold, generating a triggered action potential (4,5). Under the right conditions, triggered arrhythmias may result as the final outcome at the whole heart level.

4. CONNEXINS, GAP JUCTIONS AND CELL-CELL COMMUNICATION

In most living tissue, cells communicate by regulating conductances of ions, second messengers and small metabolites through gap junctional proteins called connexins, allowing rapid cell-to-cell communication (6-8). Connexin isoforms identified in the heart include Cx-40, Cx-43 and Cx-45 depending on tissue location (9-11). Although homomeric channels may form the bulk of active gap junctional channels, heteromeric forms of the channel exist and are functional (12,13). It is also known that disease changes the balance of connexin expression patterns, with a decrease in Cx-43 expression (14,15) and compensatory increase in Cx-45 expression (16). Moreover, there is a close link between changes in Cx-43 expression levels and electrical conduction disturbances in disease (17-19), demonstrating the importance of normal intercellular communication to normal electrical function. In addition, Cx-43 expression is reduced at cell ends and increases at lateral cell connections so there are important consequences of cardiac disease on connexin isoform expression levels, phosphorylation and location (18-22).

Numerous studies have demonstrated gap junction formation and conductances in cell pairs (23-25) so it is quite clear how intercellular communication is accomplished between cardiac cells. The question then arises: how can an inherently intracellular event (spontaneous Ca^{2+} release from the SR) cause the spread of excitation across the intact heart?

5. Ca^{2+} SPREAD BETWEEN CARDIAC MYOCYTES IN CULTURE AND CELL PAIRS

Early experiments using myocyte pairs demonstrated low resistance junctions to the passage of electrical current (26). Gap junctions are regulated by hydrogen ions, cyclic 3',5'-adenosine monophosphate (probably through channel phosphorylation) and Ca2+, among other ligands (27), making them vitally important to the maintenance of cell viability during both acute and chronic disease states.

Aside from its ability to regulate gap junctional conductance, there have been numerous studies reporting the ability of Ca^{2+} itself to move between cardiac cells via gap junctions. Suadicani *et al.* compared neonatal cardiac myocytes from wild type (WT) and Cx-43 knock-out mice (KO) and found that the intercellular movement of Ca^{2+}

also occurs primarily via gap junctions (28). Mechanical induction of Ca^{2+} waves caused slow conduction of Ca^{2+} waves (14μ/sec) across 80% of myocytes across the entire WT culture, with a loss of 87% of transmitted Ca^{2+} waves between cultured neonatal KO myocytes. Fewer myocytes showed Ca^{2+} waves in KO than WT when type 2 purinergic receptors were blocked, suggesting the involvement of intracellular ATP release in regulation of gap junctional conductances and a mechanism for reduced intercellular communication in ischemia. The authors concluded that the slow propagation velocity occurred because of poor SR organization in neonatal myocytes (29,30) as well as poor organization of connexins in the intercalated disk (31).

Cell pairs from adult heart have also served as one of the major models for the study of intercellular Ca^{2+} flow. One of the first applications of laser scanning confocal microscopy demonstrated that Ca^{2+} waves propagated with little delay between guinea pig ventricular myocyte pairs (32). In contrast, cell pairs taken from rabbits (7) showed no indication of intercellular Ca^{2+} flow under physiological conditions despite intracellular Ca^{2+} wave propagation under conditions of SR Ca^{2+} overload.

6. Ca^{2+} SPREAD BETWEEN CARDIAC MYOCYTES IN INTACT TISSUES AND WHOLE HEART

In intact myocardium, Minamikawa *et al.* were the first to demonstrate that Ca^{2+} waves occur in perfused whole rat hearts in the subepicardium (33). They periodically saw Ca^{2+} waves during physiological extracellular Ca^{2+} perfusion in a paced and arrested heart, which occurred more frequently when extracellular Ca^{2+} levels were increased to the point where Ca^{2+} overload was induced. They noted that Ca^{2+} waves traversed cell borders in all three dimensions and crossed to nearby cells; however, in damaged cells, intercellular Ca^{2+} propagation was absent, even though spontaneous Ca^{2+} waves did occur frequently. This observation is consistent with the fact that high intracellular Ca^{2+} can close connexin channels which is presumably a protective device that prevents Ca^{2+} dysregulation of cell functions and hypercontracture from spreading to neighboring cells (27,34).

The general requirement for Ca^{2+} overload in intercellular transmission of Ca^{2+} is similar to that in intact tissues where Ca^{2+} overload, often in the setting of cell damage, promotes wave propagation between myocytes of healthy and infarcted hearts. In fact, Ca^{2+} waves in myocytes of intact rat ventricular trabeculae caused different types of responses in downstream myocytes, including subthreshold Ca^{2+} "spritzes" as well as full-blown Ca^{2+} waves that propagated to both cell ends, but only during Ca^{2+} overload induced by elevated [Ca2+] in the external solution (8). The likelihood of wave transmission increased with β-adrenergic stimulation and decreased when cells were uncoupled with heptanol. However, propagation increased during Ca^{2+} overload induced by tissue damage when more waves occurred. These investigators also reported that occasional transverse wave propagation also occurred in these conditions, although most wave propagation occurred from cell ends, which is consistent with the idea that Ca^{2+} accumulation occurs normally in the intercalated disks, increasing the probability of spontaneous Ca^{2+} release from that site (7). These cellular regions have a particularly high density of gap junctions which might contribute to a higher likelihood of both Ca^{2+} accumulation and spontaneous Ca^{2+} release from these sites. Finally, although Ca^{2+} waves did propagate between cells in this study, propagation was slow, requiring hundreds of milliseconds to fully evoke a wave in a neighboring cell.

Among the most extensive studies demonstrating Ca^{2+} wave transmission are those performed in damaged ventricular trabeculae. Studies in rat trabeculae focused on the generation of Ca^{2+} waves (35,36) induced by simulation of either local mechanical or ischemic myocardial damage or by using regional application of caffeine, low Ca^{2+} concentration, or 2,3-butanedione monoxime. Each condition showed that non-uniform ECC can produce Ca^{2+} waves that underlie triggered propagated contractions, presumably as a result of passive stretch in damaged or weak myocardial regions causing release of Ca^{2+} from myofilaments which then causes a rise in cytoplasmic [Ca2+] that is late enough to induce triggered SR Ca^{2+} release in the form of Ca^{2+} waves. These authors proposed that the early release of Ca2+ from damaged cells causes Ca2+ waves that propagate across the myocardium that could produce triggered beats in the setting of non-uniform contraction in heterogeneously damaged myocardium (35-38). This proposition is particularly interesting because they documented a wide range of propagation velocities for large and small waves (ranging from 0.5-1.5mm/sec) and distances of propagation up to 450 microns (□□), making this behavior in damaged myocardium more likely to recruit enough tissue to produce a triggered beat than in nearly all other experimental models reported to date.

We have also observed the propagation of Ca^{2+} waves between myocytes of intact rat heart (39) although these events are extremely rare and usually involve only a single neighboring myocyte. Only rarely have we observed Ca^{2+} spread across multiple cells and this can take up to hundreds of milliseconds to occur. Figure 1A shows an example of Ca^{2+} wave propagation between adjoining myocytes in an intact heart from a spontaneously hypertensive rat following rapid pacing. A Ca^{2+} wave signals spontaneous Ca^{2+} release in a cell near the center of the field of myocytes (6^{th} myocyte from the top, cells separated by white horizontal lines). There is a slow cascade of Ca^{2+} waves toward the cells at the top of the image, with successive activation spreading from cell-to-cell. Figure 1B shows an expanded image (taken from the red bar to the right of the image in Figure 1A) in which Ca^{2+} "spritzes" (subthreshold Ca^{2+} transmission to downstream cells) can be seen to activate waves in adjoining cells (red arrows). We have seen this behavior very rarely in intact rat hearts (and never in guinea pig hearts) and it is consistent with other reports of this behavior in diseased myocardium, where it has been reported to occur only occasionally

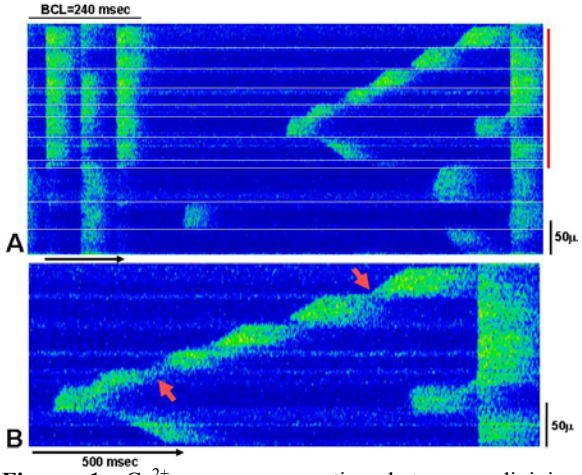

Figure 1. Ca^{2+} wave propagation between adjoining myocytes in an intact heart of a 9month old spontaneously hypertensive rat. A: Linescan confocal image (1.92msec/line) of Ca^{2+} transients recorded transversely across 11 myocytes (separated by horizontal white lines) during a pause following pacing at a basic cycle length of 240msec. The 6th cell from the top of the image developed a spontaneous Ca^{2+} wave that cascaded to the top 5 cells in succession and to one additional cell below where it was extinguished. B: higher magnification of the image (from red line to the right of the image in A) showing Ca^{2+} spritzes (red arrows) that caused propagation of Ca^{2+} waves to neighboring myocytes.

Experiments by Kaneko *et al.* (3) also used rat whole heart subepicardial myocardium to monitor the incidence of Ca^{2+} waves. They simulated Ca^{2+} overload conditions through the induction of mechanical damage by microelectrodes to observe how this damage influences Ca^{2+} wave propagation and found that intercellular wave propagation involved primarily transverse (side-to-side) Ca^{2+} spread to as many as 4-7 adjacent cells. In addition, they found that only about 20% of Ca2+-overloaded myocytes had waves that crossed cell borders. More recently, similar experiments in rat heart confirmed intercellular propagation of Ca^{2+} waves but found that Ca^{2+} waves spread to at most 2-3 cells (40).

Thus, there are some reports of the spread of Ca^{2+} waves between cells of intact tissue under Ca^{2+} overload conditions, although spread involves only a few layers of neighboring myocytes and takes a relatively long time to occur. There seems to be a somewhat different situation in non-uniformly damaged tissue where the spread of Ca^{2+} waves may be sufficient to induce triggered beats (4,41), but this appears to be a special situation. In contrast, it appears that, under most normal and pathophysiological conditions, it is difficult for intact myocardium to meet the spatial and temporal conditions required for activation of triggered activity (discussed below).

7. MECHANISMS AND REGULATION OF INTERCELLULAR Ca^{2+} WAVE PROPAGATION

If intercellular flow of Ca^{2+} is to occur between myocytes, Ca^{2+} will propagate through gap junctions either longitudinally between cell ends at the intercalated disks or laterally between cells connected side-to-side. Gap junctions are more heavily concentrated at cell ends than along cells connected laterally in normal tissue (31,42). In fact, cell-end gap junction proteins, in particular Cx-43, account for approximately 80% of all gap junction proteins within cardiac cells, as seen in sections of canine myocardium. It is thought that gap junctions are less likely to be concentrated laterally because they would have difficulty enduring the shear movements produced by cell contractions (31).

Certain factors are known to influence the likelihood and occurrence of intercellular movement of Ca2+. The most notable ones include gap junction conductivity, the amount of tissue damage caused by myocardial infarction or hypertrophy, and the ability of Ca^{2+} waves to become repetitive within a given myocyte (43,44). In regard to gap junction conductivity, experimental evidence suggests that Ca2+-overloaded regions tend to be less conductive and that phosphorylation of Cx-43 is critical to Ca^{2+} wave propagation (2,3).

It is well known that intercellular Ca^{2+} waves may be responsible for the depolarizations that cause triggered arrhythmias (4,35,45). Many common arrhythmias occur as a result of structural heart disease, such as left ventricular dysfunction and/or the presence of myocardial infarction and scar formation (46). However, it is also known that Ca^{2+} overload-induced waves promote cell depolarization in non-uniform heart tissue, which can then lead to both cellular contraction and triggered arrhythmias (3). To achieve an effective increase in the Ca^{2+} load, action potential initiation from a delayed afterdepolarization in the whole heart requires the generation of spontaneous Ca^{2+} waves in multiple, closely located cells or that a single Ca^{2+} wave quickly propagate across cell borders (47). Given that the results described in most studies found both a low incidence and a low propagation distance of Ca^{2+} waves, it is unlikely that Ca^{2+} waves alone may serve as a frequent cause of triggered cardiac arrhythmias.

It is important to note, however, that hyperphosphorylation of RyR receptors, such as occurs in heart failure (HF), causes Ca^{2+} leak from the SR. Therefore, there is a decreased Ca^{2+} load of the SR, which is commonly observed in heart failure (48,49). This increased SR leak is responsible both for reduced contractile force and potentially the increased risk of spontaneous diastolic Ca^{2+} release which could cause triggered arrhythmias (48,49). This occurs partly as the result of the fact that the electrogenic Na+ -Ca^{2+} exchanger is upregulated in many models of heart failure which promotes membrane depolarization and resulting triggered activity. In fact, the incidence of Ca^{2+} waves is increased in both overt HF and during the development of HF (39,50). Despite reported changes in connexin distribution and many additional changes in ion channels expression and distribution, t-tubule disruption, cell/tissue hypertrophy and many additional changes in cell structure and function, however, there have been no systematic studies of Ca^{2+} wave

propagation in HF models. It may be that this and other pathophysiological conditions might influence intercellular Ca^{2+} diffusion and promote the activation of Ca^{2+} waves from cell-to-cell through diseased tissue.

8. INTERCELLULAR Ca^{2+} WAVE PROPAGATION AND TRIGGERED ARRHYTHMIAS

In consideration of previous studies on intercellular Ca^{2+} movement in myocytes, it is clear that intercellular Ca^{2+} diffusion in ventricular tissues does indeed occur in some species and under a variety of experimental conditions, although results are not consistent even in similar tissues. However, despite evidence that this phenomenon can and does indeed occur, the fact that Ca^{2+} diffusion and subsequent activation of Ca^{2+} waves only affects a very few adjoining cell layers makes it very unlikely that the spread of Ca^{2+} waves can involve enough tissue to induce a triggered beat. It has been difficult to obtain accurate estimates based on experimental results of the liminal tissue mass required but estimates range from tens of thousands to nearly a millions cells (51,52) depending on whether the tissue is 2- or 3-dimensional, far exceeding the number of cells observed experimentally to be involved thus far. In contrast, the estimate of the liminal length in Purkinje fibers is far less (about 0.2mm, 53), probably involving tens to hundreds of cells) because of the 2-dimensional architecture of these cable-like structures, bringing the experimental observation closer to the theoretical requirement.

Aside from the spatial requirements, however, the temporal requirements are also critical. It is not sufficient to simply have enough cells produce Ca^{2+} waves. They must do so nearly simultaneously in order to provide enough inward depolarizing current to achieve threshold (54). Very few of the studies summarized here demonstrated rapid enough intercellular Ca^{2+} movements to provide sufficient current density over a short enough period of time to overcome the passive membrane properties that maintain the resting potential. It is the integrated depolarizing influence among many cells over a short period of time that determines the efficacy of the stimulus, requiring nearly simultaneous depolarization to produce the most effective influence in bringing the tissue to threshold, at least in normal tissue. Furthermore, the fact that there is evidence for preferential wave propagation in the longitudinal direction compared to the transverse direction would mean that wave spread could occur preferentially in only one dimension with wave propagation left to occur much more slowly in the other two dimensions, creating an enormous spatiotemporal mismatch that is likely to discourage uniform waves and depolarization in a local mass of tissue. We have recently reported that the alignment of spontaneous release in time occurs as a natural consequence of accelerated SR Ca^{2+} uptake during Ca^{2+} overload (54). This explains the temporal organization of spontaneous Ca^{2+} release in intact myocardium, at least in normal hearts. It is important to recognize that these conditions may vary in different disease states, such as heart failure, where both passive and active membrane properties of the tissue could make it easier to meet the temporal and spatial requirements for triggered activity. These changes include increasing cell size during hypertrophy, inducing levels and location of gap junctions (lateral localization rather than at cell ends), altered ion channel expression, loss of t-tubule organization and so forth. Furthermore, it is not yet known if these and other changes occur in Purkinje cells in diseased hearts where the properties of a 2-dimensional cable already probably favor triggered beat formation. The outcome of tissue remodeling in disease may thus serve to close the gap between physiological behavior and theoretical requirements for triggered activity. Future research should focus on the direction of intercellular Ca^{2+} movement (transverse vs. longitudinal), wave velocity, the distribution of gap junctions, the distance Ca^{2+} waves can travel and the conditions that cause Ca^{2+} waves to traverse cell borders, including comparisons between normal and diseased tissues and between conducting tissue and working myocardium. Clarifying the mechanisms and causes of successful intercellular Ca^{2+} flow could be instrumental in developing new therapeutic strategies in the treatment of triggered arrhythmia.

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