Calcium signalling during excitation-contraction coupling in mammalian atrial myocytes

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Summary

Atrial cardiomyocytes make an important contribution to the refilling of ventricles with blood, which enhances the subsequent ejection of blood from the heart. The dependence of cardiac function on the contribution of atria becomes increasingly important with age and exercise. We know much less about the calcium signals that link electrical depolarisation to contraction within atrial myocytes in comparison with ventricular myocytes. Nevertheless, recent work has shed new light on calcium signalling in atrial cells. At an ultrastructural level, atrial and ventricular myocytes have many similarities. However, a few key structural differences, in particular the lack of transverse tubules ('T-tubules') in atrial myocytes, make these two cell types display vastly different calcium patterns in response to depolarisation. The lack of T-tubules in atrial myocytes means that depolarisation provokes calcium signals that largely originate around the periphery of the cells. To engage the contractile machinery, the calcium signal must propagate centripetally deeper into the cells. This inward movement of calcium is ultimately controlled by hormones that can promote or decrease calcium release within the myocytes. Enhanced centripetal movement of calcium in atrial myocytes leads to increased contraction and a more substantial contribution to blood pumping. The calcium signalling paradigm within atrial cells applies to other cardiac cell types that also do not express T-tubules, such as neonatal ventricular myocytes, and Purkinje cells that aid in the spread of electrical depolarisation. Furthermore, during heart failure ventricular myocytes progressively lose their regular T-tubule expression, and their pattern of response resembles that of atrial cells.

Key words: Atrial myocytes, Ca^{2+} signalling, EC coupling, T-tubules

Physiological role of atrial myocytes

The heart undergoes a cycle of events that cause blood to be propelled to the lungs and the body. These events can be divided into two stages: diastole and systole. During the diastolic stage, the atrial and ventricular myocytes are relaxed. Systole refers to the period of contraction and consequent ejection of blood from the ventricles to the pulmonary artery or aorta. The cardiac cycle is initiated by the sinoatrial node – a group of specialised non-contractile cardiac myocytes positioned in the wall of the right atrium. All of the cells in the heart have an intrinsic ability generate action potentials (electrical impulses). However, the sinoatrial node acts as the primary pacemaker because its cells naturally discharge at a high rate, and so override the other potential pacemaking sites. Hormonal modulation of the sinoatrial node is one of the principal mechanisms for altering the frequency of the heartbeat. From the sinoatrial node, the action potential spreads over the atria causing them to contract and push blood into the ventricles. As the wave of depolarisation sweeps across the heart, it reaches the atrioventricular node, which filters and relays the signal to the ventricles via specialised conduction tissue including the Purkinje fibres. The atrial chambers contract and relax before the ventricular systole, and their activation is evident as separate electrical activity in an electrocardiogram. The time course of contraction is marginally shorter in atria compared to ventricles. Both cell types reach peak contraction within a few tens of milliseconds (Luss et al., 1999).

The ventricles contract more forcefully than the atrial chambers and are predominantly responsible for forcing blood out of the heart. However, atria can play a significant role in altering the amount of blood that loads into the ventricles before to systole. When a person is at rest, the contribution of atria to the filling of ventricles with blood is relatively low. The majority (~90%) of ventricular refilling occurs as a consequence of the venous pressure of blood returning to the heart. However, if the heart rate increases, such as during exercise or stress, then atrial contraction can account for ~20-30% of the volume of blood in the ventricles. This contribution to ventricular refilling is known as 'atrial kick' (Lo et al., 1999). Under some pathological conditions (such as mitral valve stenosis) and during ageing, the dependence on atrial kick can be critical (Nicod et al., 1986).

A common form of cardiac dysrhythmia known as 'atrial fibrillation' occurs when electrical impulses do not solely arise from the sinoatrial node, but instead spontaneously occur with high frequency from sites around the atria (~350 discharges per minute, compared with the normal sinoatrial rhythm of 60-80 beats per minute). The rapid and irregular electrical discharges during atrial fibrillation cause the atria to quiver and thereby

prevent coordinated contraction. Under these conditions, atrial kick is missing and up to one-third of the blood pumping capacity of the heart is lost (Alpert et al., 1988). Atrial fibrillation is not immediately life threatening because the pressure of blood returning to the heart can still refill the ventricles, albeit to a lesser degree. However, concomitant with the loss of pumping capacity during fibrillation, blood can pool and clot within the atria. If a blood clot travels to the brain and becomes lodged in an artery it can result in a stroke. The incidence of atrial fibrillation increases with age, and there is an increased risk of stroke in people with atrial fibrillation (Wolf et al., 1998). It is therefore clear that co-ordinated atrial function is very important.

Ca^{2+} is a ubiquitous intracellular messenger

Cells use a variety of mechanisms to sense extracellular cues and transduce them into physiological actions. Alteration of the cellular Ca^{2+} concentration is an important transduction mechanism (Berridge et al., 2000; Bootman et al., 2001a). Indeed, Ca^{2+} is an almost ubiquitous messenger that has been demonstrated to regulate activities ranging from fertilisation to cell death (Berridge et al., 1998; Bootman et al., 2001b). The ability of Ca^{2+} to regulate such a wide assortment of processes is due to the array of mechanisms that cells use to modulate the duration, amplitude, frequency and spatial extent of Ca^{2+} signalling events (Thomas et al., 1996). For example, Ca^{2+} signals can occur over microseconds to trigger activities such as exocytosis (Bootman et al., 2001b; Neher, 1998). Alternatively, they can repeat over minutes or days to control cell growth (Berridge et al., 2003). Ca^{2+} transients can occur in nano- or microscopic domains (Bootman et al., 2001b; Rizzuto and Pozzan, 2006), be sequestered into organelles (Duchen, 2000), or pass through the cytoplasm and produce a global whole-cell Ca^{2+} signal. In addition, Ca^{2+} can co-ordinate the activity of neighbouring cells by diffusing through gap junctions (Paemeleire et al., 2000) or causing the paracrine release of messengers (Charles, 2005).

The versatility of Ca^{2+} signalling derives from a complex interplay of mechanisms that either induce or reverse rises in cellular Ca^{2+} concentration. Cells use an assortment of pathways to promote Ca^{2+} signals (Bootman et al., 2002), and an equally broad panoply of systems that terminate Ca^{2+} transients and restore tissues to their resting state (Berridge et al., 2003). The net result of these 'on' and 'off' mechanisms determines the nature of the Ca^{2+} event. Since different cell types display unique combinations of such on and off mechanisms, Ca^{2+} signals are tissue specific and compatible with the particular function of each cell. Furthermore, the proteomes responsible for cellular Ca^{2+} signalling are highly plastic and can be remodelled through Ca^{2+}-dependent transcriptional/translational control mechanisms (Berridge et al., 2003).

Ca^{2+} and cardiac EC coupling

Ca^{2+} is a critical regulator of cardiac function, because it links the electrical depolarisation of cardiomyocytes with contraction (excitation-contraction coupling; EC coupling) (Bers, 2002). Elevation of Ca^{2+} within cardiac myocytes allows the actin and myosin contractile filaments to engage and slide past each other, thus shortening the cells and producing the force to propel blood. Ca^{2+} signals in cardiomyocytes are initiated as the action potential from the sinoatrial node sweeps over the heart. The consequent cellular depolarisation activates voltage-operated Ca^{2+} channels (VOCCs), which allows Ca^{2+} to flow across the sarcolemma and into the cytoplasm. This Ca^{2+} influx occurs into a narrow (~10 nm) cleft (often referred to as the 'dyadic' or 'tryadic' cleft) (Ayettey and Navaratnam, 1978), which is formed by the sarcolemma and the membrane of the internal Ca^{2+} store (the sarcoplasmic reticulum; SR) (Fig. 1). The SR is studded with Ca^{2+} release channels known as ryanodine receptors (RyRs), which are activated by Ca^{2+} itself, in a process called Ca^{2+}-induced Ca^{2+} release (CICR) (Fill and Copello, 2002; Meissner, 2004; Roderick et al., 2003). The density of RyRs is particularly high at the clefts, where the SR and sarcolemma are in close apposition, and they are therefore located directly opposite VOCCs. When Ca^{2+} floods into the cytoplasm through VOCCs it does not have far to diffuse before encountering RyRs. The rise in Ca^{2+} concentration on the cytosolic side of the RyRs causes them to open, thereby releasing Ca^{2+} sequestered within the SR and greatly amplifying the cytosolic Ca^{2+} signal. The Ca^{2+} concentration within this small space cannot be measured at present, but using mathematical modelling it has been estimated that the Ca^{2+} rise may exceed several millimolar in the centre of the cleft (Jones et al., 2006; Peskoff and Langer, 1998).

The essential effect of CICR in the dyadic cleft is to cause microscopic Ca^{2+}-release events termed 'Ca^{2+} sparks' (Cannell et al., 1995; Cheng et al., 1993). These tiny Ca^{2+} signals arise from the concerted activation of a cluster of RyRs. It has been suggested that as few as ~15 RyRs underlie Ca^{2+} sparks (Soeller and Cannell, 2002), although the variability in their time course and amplitude suggests that the number and/or cooperation between channels can vary (Parker and Wier, 1997; Shen et al., 2004). The Ca^{2+} entry signal that activates the RyR cluster probably arises from a smaller group of closely apposed VOCCs (Inoue and Bridge, 2003). Depending on the condition of the myocyte, Ca^{2+} sparks can represent an amplification of ~tenfold or more over the Ca^{2+} flux that originally entered through the VOCCs. This amplification often referred to as the 'gain' in EC coupling. The degree of gain can be altered by environmental conditions, as described below.

Eventually, Ca^{2+} ions diffuse out of the cleft and trigger contraction by binding to troponin C within the myofibrils. Ca^{2+} recovers back to resting (diastolic) levels owing to sequestration in the SR by an ATP-dependent enzyme known as SERCA (sarcoendoplasmic reticulum Ca^{2+} ATPase), and transport across the sarcolemma via the action of a Na^+/Ca^{2+} exchanger. Myocytes also posses a sarcolemmal Ca^{2+} ATPase, but this is believe to make a lesser contribution to Ca^{2+} extrusion (Eisner and Sipido, 2004).

The cardiomyocytes within different regions of the heart vary in morphology, ultrastructure and molecular composition. Nevertheless, the process described above is used to initiate Ca^{2+} signals within all the electrically excitable cardiomyocyte cell types. Fig. 2 shows some of the structural aspects of ventricular, atrial and neonatal cardiac myocytes. A striking feature of these cells is their regular striations, evident from staining for structural proteins (e.g. α-actinin, an actin-binding protein found at Z-lines; Fig. 2Ai-Ci) Ca^{2+}-release channels (RyRs; Fig. 2Aii-Cii) and plasma membrane proteins (the Na^+/Ca^{2+} exchanger; Fig. 2Aiii). The stereotypic locations of

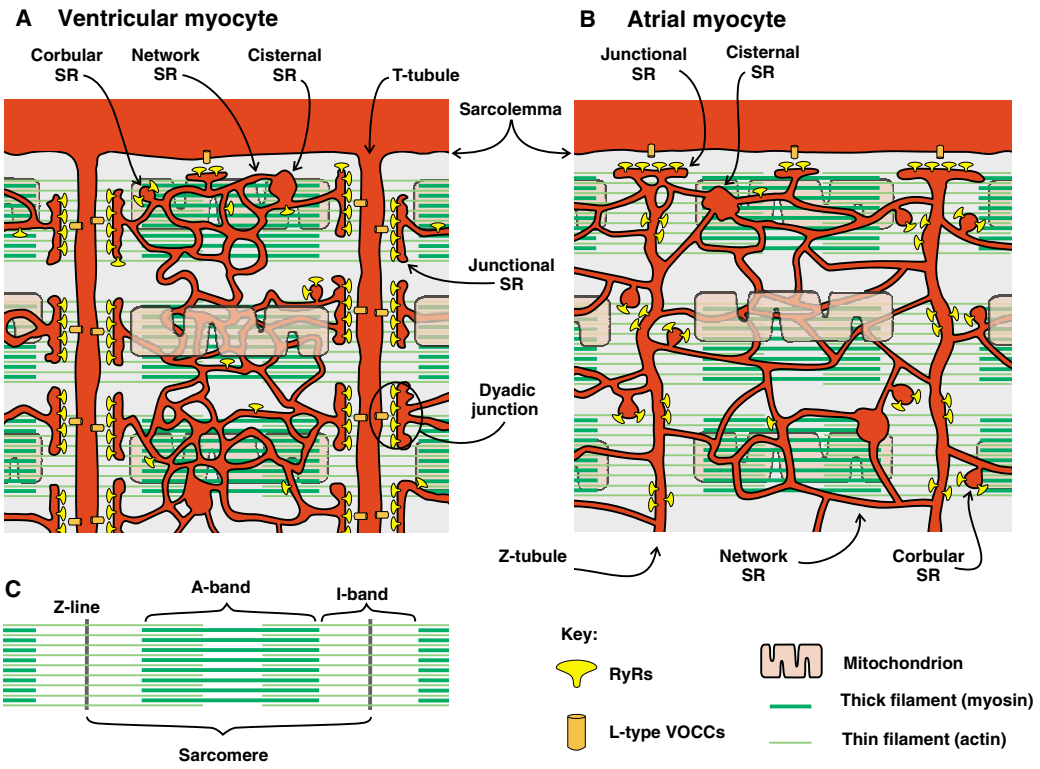

Fig. 1. Structure of ventricular, atrial and neonatal rat cardiac myocytes. Panels A and B depict portions of a ventricular and an atrial myocyte, respectively. They illustrate the relative positioning of some of the key elements involved in EC coupling. The network SR elements wrap around both the myofibrils and mitochondria. For additional representations of these cells see Franzini-Armstrong et al. and others (Franzini-Armstrong et al., 2005; Sommer and Jennings, 1992; Yamasaki et al., 1997). Panel C shows the topology of a myofibril relative to the ventricular myocyte section above it in A. The junctional couplings involving the peripheral sarcolemma in the middle of the A-band of ventricular myocytes has clearly been visualised in some studies (Chen-Izu et al., 2006) but are not always evident (Fig. 3Aii). In atrial cells, it appears that junctional coupling exists at the Z-lines and also in the middle of the A-band (Fig. 3Bii).

structural and signalling proteins within a particular cardiac cell type means that depolarisation-induced Ca^{2+} signals are reasonably consistent for that tissue. However, the variation in cellular ultrastructure between different tissues within the heart gives rise to Ca^{2+} signals with dissimilar spatial and temporal properties.

Ventricular myocyte EC coupling

Cardiac Ca^{2+} signalling has been most extensively studied in myocytes isolated from the ventricles. It is well established that Ca^{2+} signals in mammalian ventricular myocytes take the form of homogenous whole-cell increases (Cannell et al., 1995; Guatimosim et al., 2002). Such global Ca^{2+} responses reflect the spatial and temporal summation of signals from many Ca^{2+} spark sites, which are synchronously recruited during depolarisation. The critical ultrastructural detail of mammalian ventricular myocytes that promotes homogenous global Ca^{2+} transients is the presence of an extensive 'transverse tubular' system (T-tubules) (Song et al., 2005). These narrow (average diameter ~200 nm) inwardly directed projections of the sarcolemma arise at each of the Z-lines within cardiac muscle and have a regular spacing (~1.8 μm) (Brette and Orchard, 2003) (Fig. 1). The principal tubules running perpendicularly into the cells can also bear branches, giving rise to a complex network of longitudinal and axial tunnels (Ayettey and Navaratnam, 1978). The typical striated distribution of ventricular myocyte T-tubules is shown in Fig. 2Aiii, which depicts the distribution of the sarcolemmal Na^{+}/Ca^{2+} exchanger. Many of the proteins involved in generating and reversing Ca^{2+} signals are concentrated at the T-tubules (Song et al., 2005). In particular, T-tubules serve to bring VOCCs and RyRs into close proximity. Indeed, immunostaining ventricular myocytes for VOCCs and RyRs reveals that these proteins largely appear in discrete clusters within a 3-dimensional matrix throughout the cells (Chen-Izu et al., 2006; Mackenzie et al., 2004a) (Fig. 3Ai-iii). The clusters of VOCCs and RyRs have substantially overlapping distributions, indicating that they are in very close proximity (Scriven et al., 2002). When ventricular myocytes are depolarised, the action potential is relayed to VOCCs within the T-tubule network. This means that even those Ca^{2+} spark sites deep within a ventricular cell are recruited during EC coupling, thereby giving rise to a synchronised global Ca^{2+} signal (Brette et al., 2005). If ventricular myocytes are imaged at high speed during the onset of EC coupling, the Ca^{2+} signal can be seen to arise with a striated appearance (Cleemann et al., 1998; Tanaka et al., 2003). This reflects the initiation of Ca^{2+} release adjacent to the T-tubules before the Ca^{2+} signal has managed to diffuse from the dyadic junctions (Isenberg et al., 1996).

Atrial myocyte EC coupling

Unlike ventricular myocytes, atrial cells do not possess an

extensive T-tubule system (Fig. 2Biii; the Na^+/Ca^{2+} exchanger immunostaining is around the outside of the cell only), although some atrial cells possess a more rudimentary transverse-axial tubular network (Ayettey and Navaratnam, 1978; Brette and Orchard, 2003; Gotoh, 1983; Tidball et al., 1991). In place of the T-tubules, atrial cells have prominent SR elements, which have been described as 'Z-tubules' (Fig. 1) (Yamasaki et al., 1997). Just like T-tubules, these structures are perpendicular to the long axis of the cells. Atrial cells therefore contain a form of transversely oriented tubule, but it is formed from internal SR membrane and not the sarcolemma.

The SR in cardiac muscle has a highly convoluted organisation that can be classified into four major structural elements: junctional, corbular (*corbula* means 'little basket' in Latin), cisternal and network (Fig. 1). Both ventricular and atrial cells possess all these SR forms, but their relative abundance is different (Yamasaki et al., 1997). For example, ventricular myocytes have a more extensive network SR running alongside the myofibrils and interweaving with the mitochondria. By contrast, atrial myocytes have a less complex network SR, but substantially more corbular compartments (Franzini-Armstrong et al., 2005; Sommer and Jennings, 1992). The corbular SR possesses both RyRs and the Ca^{2+} storage protein calsequestrin (Jorgensen et al., 1993) and is therefore a potential source for amplification of Ca^{2+} responses by CICR. Owing to its abundance in atrial myocytes, the corbular SR may contribute significantly to the generation of Ca^{2+} signals.

The pattern of RyR localisation in atrial myocytes has some similarity to that observed in ventricular cells, in that most RyRs lie within regularly spaced transverse striations corresponding to the positions of the Z-tubules (Figs 2Bii and 3Bii). However, there is one crucial difference between atrial and ventricular myocytes: that is the expression of additional RyR clusters around the periphery of the atrial cells (Carl et al., 1995; Hatem et al., 1997; Mackenzie et al., 2001) (Fig. 2Bii). Atrial myocytes therefore possess two populations of RyRs. One minor group (junctional RyRs), sit just beneath the sarcolemma. The other channels (non-junctional RyRs) are deeper inside the cell and constitute the bulk of the RyR population. Although the junctional RyRs represent a small fraction of the total number of channels, they are crucially important in atrial EC coupling (see below).

The localisation of VOCCs is entirely different in ventricular and atrial myocytes. Because of the general lack of T-tubules, the sarcolemma does not regularly protrude into atrial cells, and VOCCs only function around the outside of the myocytes (Mackenzie et al., 2004a). As a consequence, the close apposition of RyRs and VOCCs that is necessary for triggering EC coupling occurs only at the cell periphery (Fig. 3Bi-iii).

These differences in ultrastructure have a significant effect on the spatial properties of the Ca^{2+} signals. As described above, ventricular myocytes display homogenous responses,

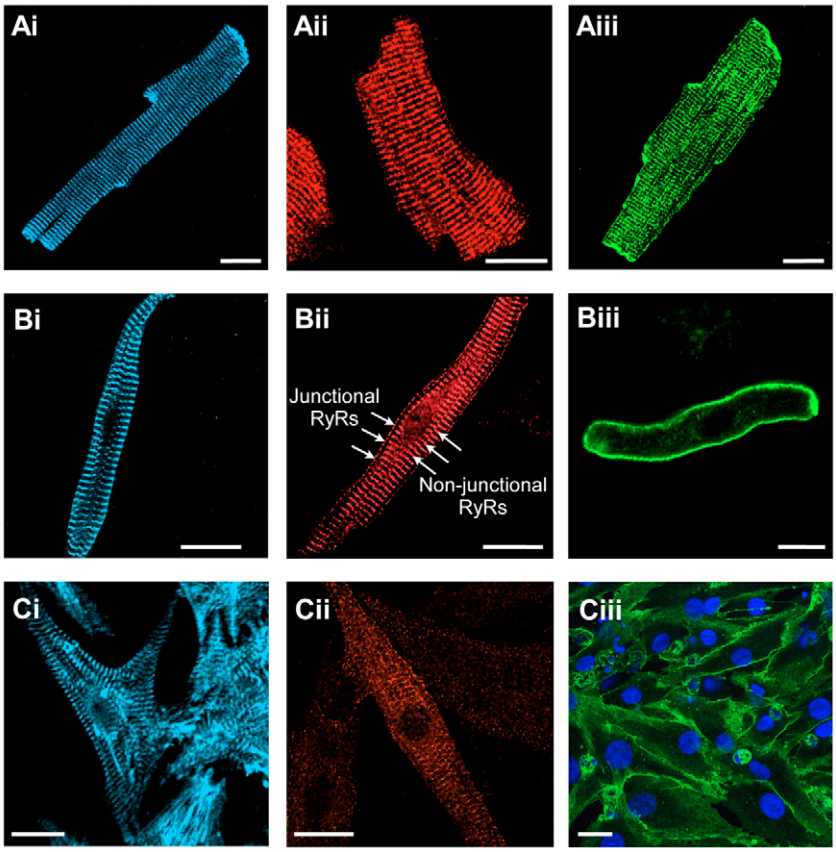

Fig. 2. Structure of ventricular, atrial and neonatal rat cardiac myocytes. Panels A, B and C depict immunostained adult ventricular, adult atrial and neonatal ventricular myocytes respectively. The left-hand panels (Ai, Bi and Ci) show cells immunostained with a monoclonal antibody raised against α-actinin (Sigma). The middle panels (Aii, Bii and Cii) display cells immunostained with a polyclonal antibody raised against type 2 RyRs (a kind gift from Prof. V. Sorrentino, San Raffaele Scientific Institute, Siena, Italy). The right-hand panels (Aiii, Biii and Ciii) depict cells immunostained with a monoclonal antibody raised against the type 1 sodium/calcium exchanger (R3F1; a kind gift from Prof. K. Philipson, UCLA, California). To visualise the NCX staining in the neonatal myocytes, a field of confluent cells is depicted (Ciii) because this provides the best contrast in these flat cells. The neonatal myocyte nuclei were visualised by DAPI staining (pseudocoloured in blue). Specific immunostaining was visualised using secondary antibodies conjugated to Alexa Fluor 405 (blue), 488 (green) or 568 (red). The α-actinin staining shows the precise striated structure of the fully differentiated adult ventricular and atrial cells (Ai and Bi). The neonatal cells show α-actinin striation (Ci), but they do not have the same degree of organisation as their adult counterparts. RyRs have a regular transverse striated pattern in adult cells (Aii and Bii). Note the two distinct populations of RyRs in adult atrial cells. In addition to the transverse 'non-junctional' RyRs, atrial cells express a ring of 'junctional' RyRs (Bii). The latter are responsible for the initiation of EC coupling around the circumference of the cell. In the neonatal myocytes (Ci), the degree of RyR expression is lower and these are less organised than in adult cells. In both atrial (Biii) and neonatal cells (Ciii), the NCX staining is prominent only around the circumference of the cells. In ventricular myocytes that have T-tubules, the NCX protein is evident on the sarcolemma and at the Z-lines deep inside the cells (Aiii). Bars, 20 μm.

which arise from the simultaneous recruitment of Ca^{2+} sparks throughout a cell. However, in atrial myocytes, EC coupling is initiated around the periphery of the cells, because this is the only place where the VOCCs and junctional RyRs come together. High-speed imaging of atrial myocyte responses has shown that multiple Ca^{2+} sparks sites are simultaneously recruited around the edge of a cell immediately after depolarisation (Kockskamper et al., 2001; Mackenzie et al., 2001). Within a few tens of milliseconds, these microscopic Ca^{2+} transients appear to spread laterally and merge, thereby causing a shell of elevated Ca^{2+} concentration beneath the sarcolemma, with no immediate response deeper inside (Fig. 4A,B). From that point, the development of the Ca^{2+} signal depends on the status of the myocytes, factors such as the Ca^{2+} content of the SR and the presence of 'positive inotropes' (agents that increase the force of heart contraction) determine whether the peripheral Ca^{2+} signal will pass further into the cells or not (Mackenzie et al., 2004a).

Under basal conditions, with cells electrically paced to their steady-state Ca^{2+} load, the Ca^{2+} signal beneath the sarcolemma does not propagate significantly into the centre of the cells (Fig. 4C) (Mackenzie et al., 2001; Mackenzie et al., 2004a). At the peak of the Ca^{2+} response, approximately 20 milliseconds after stimulation, the peripheral Ca^{2+} signal can reach ~1 μM. However, owing to the lack of inward spreading, the Ca^{2+} concentration declines sharply with distance away from the sarcolemma, and the centre of the cell barely shows an increase over the normal prestimulated Ca^{2+} level of ~100 nM. This spatially heterogeneous pattern is even maintained while the Ca^{2+} transients recover back to their pre-depolarisation (diastolic) condition (Fig. 4B). Similar results have been obtained using atrial cells from different mammalian species, including rat (Mackenzie et al., 2001; Tanaka et al., 2003; Woo et al., 2002), guinea pig (Berlin, 1995; Lipp et al., 1990), cat (Huser et al., 1996; Kockskamper et al., 2001; Sheehan and Blatter, 2003) and human (Hatem et al., 1997). These studies all agree that atrial Ca^{2+} responses originate at subsarcolemmal sites and that the Ca^{2+} signals have the greatest amplitude and rate of rise in the peripheral initiation zone. There is some disagreement about how much the subsarcolemmal Ca^{2+} signal is able to propagate into cells, but this probably reflects experimental variation in parameters such as SR Ca^{2+} content and the presence of cyclic AMP (cAMP), as discussed below.

Modulation of atrial Ca^{2+} responses

Under basal stimulation conditions, as described above, the elevation in Ca^{2+} concentration evoked by depolarisation of an atrial myocyte is largely restricted to the periphery of the cells. However, the majority of protein filaments that must sense the Ca^{2+} signal in order to cause contraction are located deeper inside the cell. What limits the inward propagation of the Ca^{2+} transient, and can it be overcome so that Ca^{2+} can reach the contractile machinery?

Given the regular distribution of RyRs inside atrial myocytes, it is perhaps a little surprising that Ca^{2+} signals can be restricted to the periphery at all. Clearly, the junctional RyRs are closest to the VOCCs, and that is why they respond. Although non-junctional RyRs are further away from the sarcolemmal VOCCs, they are not that remote. One might expect the non-junctional RyRs to sense the subsarcolemmal Ca^{2+} sparks and relay the Ca^{2+} signal deeper into the myocyte via successive rounds of CICR. Such a saltatoric conduction of Ca^{2+} waves between clusters of channels has been demonstrated to underlie the propagation of Ca^{2+} signals within cells (Bootman et al., 1997; Coombes et al., 2004; Keizer et al., 1998; Kockskamper et al., 2001). However, this centripetal movement of Ca^{2+} clearly does not occur under basal conditions.

Many studies of RyR distribution in atrial cells have shown that the junctional RyRs are separated from the non-junctional RyRs by a ~2 μm gap (Carl et al., 1995; Chen-Izu et al., 2006; Kockskamper et al., 2001; Mackenzie et al., 2001; Mackenzie et al., 2004a) (Fig. 2Bii and Fig. 3Bii). Although cytosolic Ca^{2+} can diffuse further than 2 μm, the distance is sufficiently large for the Ca^{2+} concentration to fall below that required for the activation of a Ca^{2+} spark (Izu et al., 2006). Therefore, one possibility why the subsarcolemmal Ca^{2+} signal does not always trigger centripetal Ca^{2+}

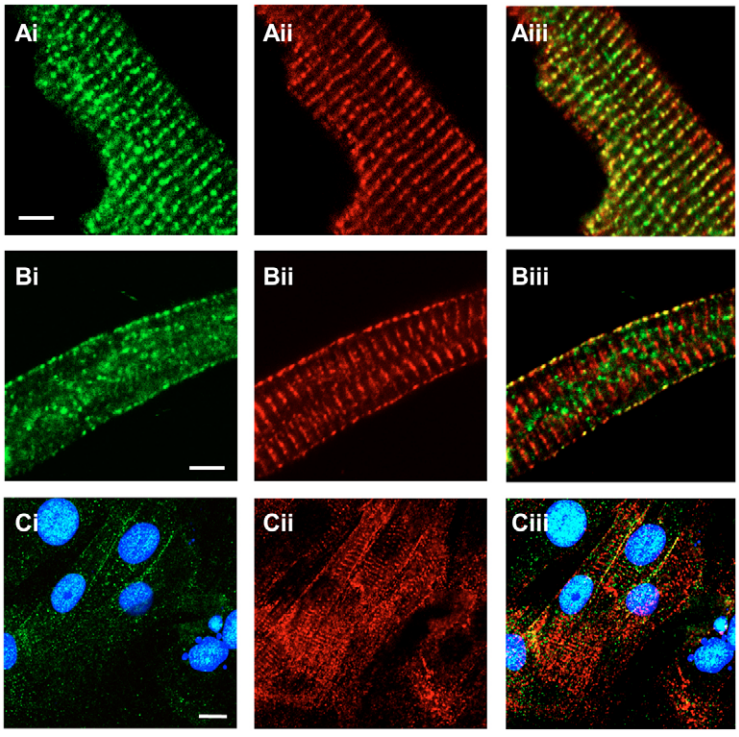

Fig. 3. Colocalisation of VOCCs and RyRs in ventricular, atrial and neonatal rat cardiac myocytes. Panels A, B and C depict immunostained adult ventricular, adult atrial and neonatal ventricular myocytes, respectively. The left-hand panels (Ai, Bi and Ci) show cells immunostained with a polyclonal antibody raised against the $\alpha 1_c$ subunit of L-type VOCCs (CNC1; a kind gift from Prof. W. Catterall, Dept. of Pharmacology, University of Washington, Seattle). The middle panels (Aii, Bii and Cii) display cells immunostained with a monoclonal antibody raised against type 2 RyRs (Calbiochem; C3-33). The right-hand panels (Aiii, Biii and Ciii) depict a merge of the VOCC and RyR images. The neonatal myocyte nuclei were visualised using DAPI staining (blue). Specific immunostaining was visualised using secondary antibodies conjugated to Alexa Fluor 488 or 568. Bars, 10 μm.

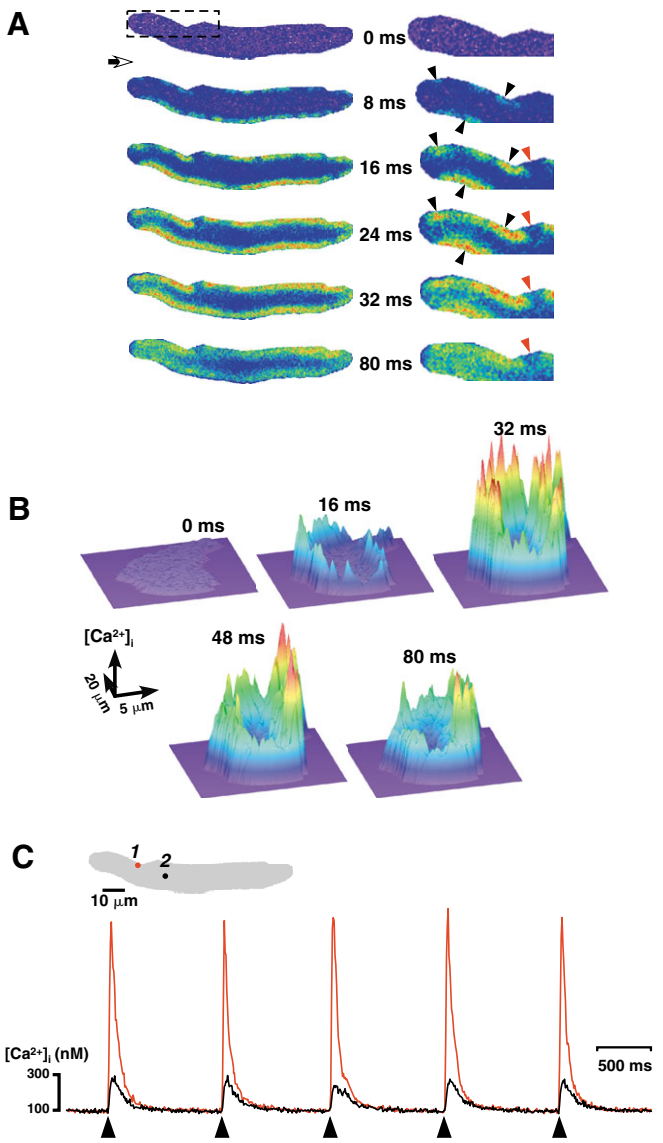

Fig. 4. Spatially heterogeneous Ca^{2+} signalling during atrial myocyte EC-coupling. Panel A shows the development of a Ca^{2+} signal following electrical depolarisation in a single atrial myocyte. The left-hand montage shows pseudocolour-coded images of the entire cell. To indicate the initiation of discrete Ca^{2+} sparks (designated by the arrowheads) and their lateral spreading, the right-hand column of images depicts part of the cell (the region bounded by the dashed box) at a higher magnification. The time at which the electrical pulse was applied is shown by the open arrow. Three obvious Ca^{2+} spark sites are marked by black arrowheads. The surface plots in panel B depict the spatial and temporal development of a Ca^{2+} signal from a different atrial cell. The Ca^{2+} concentration is indicated by both the colour and the height of the peaks (blue/green indicates low $[Ca^{2+}]$ and yellow/red depicts high $[Ca^{2+}]$). A clear peripheral ring of Ca^{2+} is evident with a steep 'valley' of low Ca^{2+} concentration in the centre of the cell. Panel C illustrates the consistency of the spatial gradient of Ca^{2+} signalling in atrial myocytes during electrical pacing. The traces show the Ca^{2+} concentration at the two regions depicted in panel A. The peripheral region (red circle and red trace) displays a large Ca^{2+} rise with each depolarisation (denoted by the black arrowheads) whereas the central region has a lower response (black circle and black trace). This figure was reproduced with permission from Blackwell Publishing and The Physiological Society (Mackenzie et al., 2001).

waves is that the gap in RyR distribution acts as a firebreak to diminish the progression of the Ca^{2+} signal. Beyond the gap, the non-junctional RyR clusters are spaced <1 μm apart in the transverse direction (they are ~2 μm apart along the longitudinal axis) (Chen-Izu et al., 2006), so that centripetal propagation is more likely once the gap has been overcome.

In addition, the junctional RyRs that underlie the peripheral Ca^{2+} signal appear to be functionally distinct from their non-junctional counterparts. For example, the frequencies of spontaneous Ca^{2+} sparks (which can be observed when myocytes are not electrically paced) are significantly greater in the subsarcolemmal region than in the centre of atrial cells (Brette et al., 2005; Lipp et al., 2000; Woo et al., 2003a). It has been proposed that junctional RyRs interact directly with the C-terminal tail of L-type VOCCs, and that this physical coupling sensitises the RyRs to Ca^{2+} and thereby promotes CICR (Woo et al., 2003b). Since the non-junctional RyRs are distant from the VOCCs, they are not similarly sensitised and thus require a higher cytosolic Ca^{2+} concentration or larger SR Ca^{2+} load before CICR is initiated (Huser et al., 1996). These putative differences in the sensitivity of junctional and non-junctional RyRs to Ca^{2+} may contribute to the spatial patterning of Ca^{2+} signals during atrial myocyte EC coupling, because CICR will be more easily activated in the junctional region than in the centre of the cells.

A further reason why centripetal Ca^{2+} waves do not always occur is the interference of Ca^{2+} movement by cellular organelles. In particular, mitochondria and SERCAs restrict the Ca^{2+} signal to the periphery of atrial myocytes under basal conditions (Mackenzie et al., 2004a). Both of these Ca^{2+} transport mechanisms provide a powerful buffer against rises of cellular Ca^{2+} concentration (Diaz et al., 2005; Gunter et al., 2004; Landgraf et al., 2004). Inhibition of either of these Ca^{2+} sequestration pathways allows the peripheral Ca^{2+} signal to propagate significantly into the interior of atrial myocytes (Mackenzie et al., 2004a). Electron microscopy and immunofluorescence studies have confirmed that mitochondria and SERCAs are closely aligned with the junctional RyRs (Kockskamper et al., 2001). It therefore appears that activation of the junctional RyRs leads to Ca^{2+} sparks that have the capacity to recruit their neighbouring non-junctional RyR clusters by CICR, but under basal conditions a firewall composed of mitochondria and Ca^{2+} pumps prevents the inward diffusion of the signal. The contractile myofilaments may also contribute to the buffering of Ca^{2+} and hinder movement of the ion within myocytes because they contain Ca^{2+}-binding proteins.

Artificially increasing the buffering capacity of the sarcoplasm within atrial myocytes by introducing exogenous Ca^{2+} chelators leads to a sharper definition of the peripheral Ca^{2+} signal and diminishes the response from non-junctional RyRs (Sheehan and Blatter, 2003). Diffusion of Ca^{2+} away from the subsarcolemmal initiation sites is therefore crucial to trigger propagating CICR deeper in the cell.

The conserved ultrastructural features of mammalian atrial myocytes dictate that the Ca^{2+} signals they produce are spatially heterogeneous. The expression of powerful Ca^{2+}

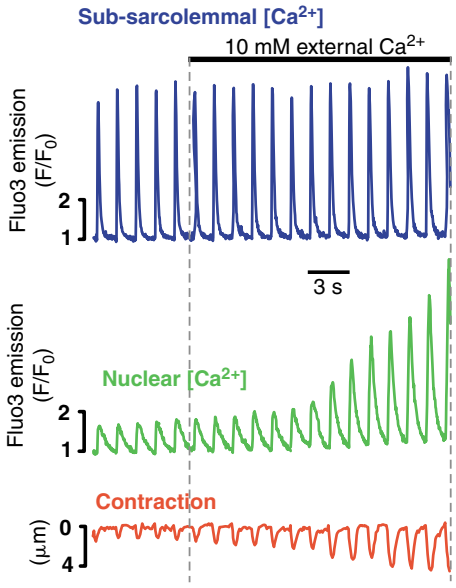

Fig. 5. Positive inotropic effect of raising extracellular Ca^{2+}. Progressive centripetal propagation of Ca^{2+} signals promotes contraction of atrial cardiomyocytes. The traces shown were obtained from a single atrial myocyte that was electrically paced and loaded with the Ca^{2+} indicator fluo3. Ca^{2+} elevation is denoted by an increase in fluo3 emission. The cell was electrically paced at a constant frequency, just as if it was receiving a regular action potential from the sinoatrial node. To simulate the effect of a positive inotropic agonist, the extracellular Ca^{2+} concentration was elevated from 1 to 10 mM as indicated. This provides a larger Ca^{2+} influx signal and ultimately leads to increased SR Ca^{2+} content, thereby mimicking the effect of β-adrenergic stimulation (Huser et al., 1996; Mackenzie et al., 2004a), but without changing the phosphorylation status of any of the proteins involved in EC coupling. The inward movement of Ca^{2+} within the cell was monitored by observing changes in nuclear Ca^{2+} concentration. Since atrial cell nuclei are centrally positioned, and nuclear Ca^{2+} derives almost exclusively from the surrounding cytoplasm, they are ideal cellular areas in which the ability of Ca^{2+} to penetrate into cells can be assessed. The change of extracellular Ca^{2+} did not affect the amplitude of Ca^{2+} signals in the sub-sarcolemmal region where EC coupling was initiated, but it caused a progressive increase in Ca^{2+} signalling in the remainder of the cell, as shown by the increased amplitude of the signals in the nuclear region. Concomitantly with greater inward movement of Ca^{2+}, the cellular contraction was enhanced.

sequestration pathways in proximity to the zones where EC coupling initiates disrupts the diffusion of Ca^{2+} from the periphery of the cells, so that the saltatoric centripetal propagation of a Ca^{2+} wave does not occur. A major function of atrial myocytes, however, is to promote the refilling of ventricles with blood, particularly under times of activity and stress. Given that the contractile machinery is deeper inside the myocytes than the junctional regions, the cells must have a mechanism to generate Ca^{2+} signals that overcome the firewall. Decreasing SERCA activity or the extent of mitochondrial Ca^{2+} uptake allows the propagation of the Ca^{2+} signal, but this does not happen in a physiological context. Instead, atrial myocytes use systems to enhance the trigger Ca^{2+} influx through VOCCs, elevate the Ca^{2+} content of the SR and increase the sensitivity of RyRs to CICR. Stimulation of any of these processes is sufficient to promote the centripetal propagation of Ca^{2+} into the cells and thereby promote contraction (Huser et al., 1996; Mackenzie et al., 2004a).

A major hormonal influence on the force of contraction of the heart occurs via the sympathetic nervous system (Bers, 2002). Stimulation of these nerves leads to the engagement of β-adrenergic receptors on the sarcolemma of myocytes, and the consequent production of cAMP and activation of protein kinase A (PKA). This kinase can phosphorylate cardiac L-type VOCCs, leading to an increase in mean channel open time and/or channel opening probability, thereby enhancing the trigger Ca^{2+} influx following depolarisation (van der Heyden et al., 2005). In addition, PKA phosphorylates phospholamban, a protein on the SR that interacts physically with the SERCAs to inhibit Ca^{2+} transport (Frank et al., 2003). Phosphorylation by PKA dissociates phospholamban from the SERCA pumps and increases Ca^{2+} sequestration, which can lead to a dramatic increase in SR Ca^{2+} content. The lumenal Ca^{2+} concentration within the SR has a substantial positive impact on the 'gain' of CICR (i.e. the ratio of the resultant Ca^{2+} signal compared with the trigger Ca^{2+} concentration). Increasing the lumenal Ca^{2+} concentration has a steep non-linear effect on the sensitivity of RyRs to CICR, and promotes more substantial Ca^{2+} flux (Bassani et al., 1995; Diaz et al., 2005; Koizumi et al., 1999; Laver, 2005). This probably occurs through saturation of the Ca^{2+}-binding protein calsequestrin, which provides a reservoir of releasable Ca^{2+} and allosterically regulates RyR opening (Beard et al., 2005; Gyorke et al., 2004). A further effect of PKA may be to phosphorylate RyRs and consequently increase their sensitivity to Ca^{2+} and enhance their open probability (Bers, 2004). Sympathetic stimulation of the heart therefore engages several molecular mechanisms to generate a positive inotropic effect.

β-adrenergic stimulation promotes the reliable centripetal propagation of the Ca^{2+} signal during EC coupling in atrial myocytes. Following a brief stimulation with a β-adrenergic agonist, the pattern of Ca^{2+} signalling changes dramatically from a peripheral ring of Ca^{2+} to a full-blown global response in which the non-junctional RyRs participate (Hatem et al., 1997). Perfusing atrial myocytes with cAMP via a patch pipette has a similar effect (Woo et al., 2002). Concomitant with the change in centripetal propagation of the Ca^{2+} signal, there is a significant increase in contraction (Mackenzie et al., 2004a). Hormones can thus control atrial contraction by modulating the spatial distribution of the Ca^{2+} signal. The interplay of several Ca^{2+} management systems dictates the spatial extent of the atrial myocyte Ca^{2+} signal. In turn, the spatial properties of the Ca^{2+} signal dictate the degree of cellular contraction. The non-junctional RyRs within atrial myocytes therefore constitute a positive inotropic reserve, which can be recruited when stronger cellular contraction is required.

In addition to RyRs, atrial myocytes express another form of intracellular Ca^{2+} release channel: inositol 1,4,5-trisphosphate receptors ($InsP_3Rs$) (Li et al., 2005; Lipp et al., 2000; Mackenzie et al., 2002). Ca^{2+} release from $InsP_3Rs$ is triggered in response to stimulation of myocytes with hormones that activate phospholipase C, such as the potent vasoconstrictive peptide hormone endothelin-1. Although $InsP_3Rs$ are ~100 times less abundant than RyRs within the heart, they appear to be able to subtly modulate cardiac functions. In particular, $InsP_3Rs$ can regulate gene transcription

and stimulate positive inotropy (Li et al., 2005; Proven et al., 2006; Wu et al., 2006). For atrial myocytes, $InsP_3R$ activation enhances both the centripetal propagation of Ca^{2+} signals and cellular contraction (Mackenzie et al., 2004a). Pharmacological or genetic ablation of $InsP_3R$ function prevents the positive inotropic effect of agonists such as endothelin-1 on atrial myocytes (Li et al., 2005; Mackenzie et al., 2004b).

$InsP_3R$ activation has also been shown to cause spontaneous Ca^{2+} signals in many studies (Mackenzie et al., 2004b; Woodcock and Matkovich, 2005). The generation of spontaneous Ca^{2+} signals within myocytes has the potential to perturb the normal beating of myocytes during the cardiac cycle. For reasons that are presently not understood, atrial myocytes express substantially more $InsP_3Rs$ than ventricular cells (Li et al., 2005; Lipp et al., 2000). This means that atrial myocytes are more prone to the potential dysrhythmic effects of $InsP_3R$ activity. However, the expression of $InsP_3Rs$ within ventricular myocytes is increased during some forms of heart failure (Go et al., 1995). In this case, ventricular myocytes will become more like atrial cells – being more responsive to $InsP_3R$ activation – and could potentially suffer from greater hormone-induced dysrhythmia.

Atrial myocytes show a spatial continuum of Ca^{2+} signals

Under basal conditions, atrial Ca^{2+} signals are restricted to the subsarcolemmal periphery of cells (Fig. 4), whereas, following application of a positive inotropic stimulus, global Ca^{2+} transients are evoked (Mackenzie et al., 2004a). These responses represent the least and greatest Ca^{2+} signals that atrial myocytes can produce. However, these cells are not digital in their patterns of Ca^{2+} response. Instead, they show a spatially graded continuum of Ca^{2+} signals, which correlate with the extent of cellular contraction. This is illustrated in Fig. 5, which shows simultaneous recordings of Ca^{2+} concentration in the subsarcolemmal and nuclear regions of an atrial myocyte, and also the corresponding degree of cellular contraction. The cell was electrically paced at a constant frequency, just as if it was receiving a regular action potential from the sinoatrial node. To simulate the effect of a positive inotropic agonist, the extracellular Ca^{2+} concentration was elevated from 1 to 10 mM as indicated. The figure shows that the switch to 10 mM Ca^{2+} causes a substantial positive inotropic effect: the ability of the Ca^{2+} signal to invade the nuclear region increases with each depolarisation and the contraction of the cell is simultaneously augmented. The progressive increase in both the centripetal propagation of the Ca^{2+} signal and contraction shows that atrial cells can smoothly grade their responses over a wide range.

Rapid central Ca^{2+} responses in atrial myocytes

In the majority of studies examining the spatial properties of atrial Ca^{2+} signalling, peripheral Ca^{2+} signals have been found to peak tens of milliseconds before the central Ca^{2+} signals fully develop (Mackenzie et al., 2004a; Sheehan and Blatter, 2003). Furthermore, it has been demonstrated that Ca^{2+} release from the junctional RyRs can be abruptly terminated by rapidly repolarising an atrial myocyte, whereas once the non-junctional RyRs are activated their Ca^{2+} release persists for a while after repolarisation (Hatem et al., 1997; Sheehan and Blatter, 2003). These observations support the concept that the junctional RyRs are under the control of membrane potential via activation of VOCCs, whereas the non-junctional RyRs are recruited by a distinct CICR mechanism. However, a few studies have demonstrated a component of fast Ca^{2+} release within central regions of atrial cells (Kirk et al., 2003; Woo et al., 2002). As mentioned above, some atrial myocytes have a rudimentary T-tubule system (Ayettey and Navaratnam, 1978; Gotoh, 1983; Tidball et al., 1991), and this may be the basis for some rapid central Ca^{2+} responses. In atrial cells that have T-tubules, electrically evoked Ca^{2+} signals originate simultaneously at multiple independent locations within the cells (Kirk et al., 2003).

Why atrial myocytes possess T-tubules at all is unclear. Since T-tubules penetrate deep into the cells, they can activate Ca^{2+} signals beyond the firewall described above. It is plausible that they serve to trigger CICR within the cell centre and thereby increase the speed at which Ca^{2+} reaches the myofilaments. In addition, by acting as a conduit for extracellular Ca^{2+}, the T-tubules may increase the SR Ca^{2+} content and speed SR refilling (Brette et al., 2005; Woo et al., 2005). These effects could lead to more homogenous Ca^{2+} transients within individual myocytes. However, the density and morphology of T-tubules not only differs between ventricular and atrial myocytes but can also vary in extent between cells of the right and left atrial chambers. Furthermore, within the different atrial chambers, the pattern of T-tubules varies from cell to cell (Kirk et al., 2003; Tidball et al., 1991; Woo et al., 2005). The inconsistency in T-tubule expression, density and morphology in atrial cells is therefore puzzling, because it could cause substantial variability in the response of adjacent cells within the working atria.

The relevance of atrial myocyte Ca^{2+} signalling to other cell types, heart failure and development

The Ca^{2+} signalling paradigm of atrial myocytes is also relevant to other tissues where excitation only occurs at the periphery of the cells. For example, cardiac Purkinje cells, which underlie the conduction of action potentials into the mass of ventricular myocytes, display patterns of Ca^{2+} signalling similar to those observed in atrial cells (Boyden et al., 2000; Cordeiro et al., 2001). Purkinje cells are non-contractile myocytes responsible for the transmission of electrical signals. Like atrial cells, they do not have T-tubules but express RyRs in regular striated patterns throughout their volume (Cordeiro et al., 2001; Stuyvers et al., 2005). Moreover, Ca^{2+} signals in Purkinje cells initiate in subsarcolemmal locations and show a weak centripetal propagation into the cell interior. Increasing the concentration of extracellular Ca^{2+} or application of a β-adrenergic agonist promotes the response of non-junctional RyRs, but the pattern of peripheral to central propagation is maintained (Cordeiro et al., 2001). The physiological consequences of modulating the centripetal propagation of Ca^{2+} within Purkinje cells are not known.

In some pathological conditions that lead to heart failure, the density of T-tubules in ventricular myocytes decreases (Balijepalli et al., 2003; He et al., 2001). Consequently, Ca^{2+} signals do not arise synchronously throughout ventricular cells, because there are cellular locations where EC coupling fails to occur (Louch et al., 2004; Song et al., 2006). A marked reduction in T-tubule density leads to non-simultaneous

recruitment of the Ca^{2+} sparks within a ventricular myocyte, which decreases the amplitude and/or rate of rise of the Ca^{2+} signal (Brette et al., 2004; Louch et al., 2004). The functional consequences of detubulation can be mimicked to an extent by pharmacological agents that decrease Ca^{2+} entry through the L-type VOCCs (Cheng et al., 1995) or reduce the activation of RyRs by CICR (Diaz et al., 2002). The loss of global VOCC/RyR activation leads to patchy Ca^{2+} signals, where parts of the myocyte respond poorly or not at all (Shiels and White, 2005). The decline in the kinetics and amplitude of the Ca^{2+} signal has a detrimental effect on cellular contraction (Gomez et al., 1997). If a substantial number of T-tubules are lost, the Ca^{2+} signal within the ventricular myocytes begins to resemble that of atrial cells, displaying a peripheral-only initiation of EC coupling. Chemically-induced detubulation (Brette et al., 2005) or maintaining ventricular cells in long-term culture (where the lack of electrical pacing causes loss of T-tubules) produces a similar effect (Lipp et al., 1996).

The Ca^{2+} signals within detubulated cells can be substantially rescued by stimulation with positive inotropes. For example, application of β-adrenergic stimuli promotes the centripetal propagation of Ca^{2+} within detubulated ventricular cells and restores the kinetics and amplitude of the Ca^{2+} signal (Brette et al., 2004). It therefore appears that when ventricular myocytes deteriorate during heart failure, the loss of T-tubules makes their pattern of Ca^{2+} signalling increasingly like that in atrial cells. They turn out to be dependent on CICR to propagate Ca^{2+} signals away from the peripheral sites where EC coupling is initiated, and the successful centripetal movement of Ca^{2+} can be reliant on the presence of positive inotropes.

T-tubules develop after birth in mammals, and there is a progressive increase in the density and complexity of sarcolemmal invaginations (Chen et al., 1995; Sedarat et al., 2000). Neonatal ventricular myocytes are therefore not the same as their adult counterparts. Neonatal cells show some degree of striation, but are still far from being fully organised. Figs 2 and 3 demonstrate the variation in shape and structure within neonatal cells and, in particular, the absence of T-tubules (Fig. 2Ciii). As for atrial myocytes, the lack of T-tubules in neonatal cells means that RyRs and VOCCs co-localise around the periphery of the cells (Fig. 3Ci-iii). Consequently, neonatal myocytes display Ca^{2+} signals that are similar to the responses seen in atrial myocytes – they originate at the subsarcolemmal sites and show a diminishing centripetal movement (Haddock et al., 1999; Perez et al., 2005). Indeed, many features of neonatal ventricular myocytes responses are just like those of adult atrial cells. For example, when stimulated with the RyR agonist caffeine, neonatal cells give homogenous Ca^{2+} signals, which indicates that they have RyRs throughout the cell and replete Ca^{2+} stores. But the bulk of RyRs deep within the myocytes do not generally participate in EC coupling. Furthermore, again similarly to the situation in atrial cells, spontaneous Ca^{2+} sparks initiate predominantly in subsarcolemmal regions of newborn cells (Haddock et al., 1999). Since, in biochemical and electrophysiological assays, the RyRs in neonatal and adult ventricular myocytes are functionally equivalent (Perez et al., 2005), the differences in the spatial pattern of Ca^{2+} signalling can largely be ascribed to the lack of T-tubules. The physiology of neonatal cells is also different from that in adult tissue, however, because the SR is less well developed and has a smaller Ca^{2+} uptake capacity. Furthermore, neonatal cells express lower levels of L-type VOCCs and RyRs (Perez et al., 2005) (Figs 2 and 3).

T-tubules are actually a feature of mammalian cells only. Ventricular myocytes from non-mammalian vertebrates do not possess these structures. Characterisation of Ca^{2+} signalling in ventricular myocytes from animals such as fish (Shiels and White, 2005) has demonstrated that their responses resemble those of mammalian atrial cells. Non-mammalian ventricular cells largely display junctional couplings of VOCCs and RyRs around the cellular periphery (Protasi et al., 1996; Sommer and Jennings, 1992). Peripheral initiation and centripetal propagation is therefore the most commonly observed cardiac Ca^{2+} signalling paradigm within biology. It occurs within the majority of mammalian cardiac cell types and is the sole mechanism underlying EC coupling in other animals.

There are also electrically excitable cells outside the heart that display peripheral initiation of Ca^{2+} signalling around the cell soma, and rely on amplification by CICR from centrally located RyRs. Neurons, for example, display such Ca^{2+} profiles (McDonough et al., 2000). These cells do not possess the regularly arranged dyadic couplings and internal Ca^{2+} stores seen in adult cardiac cells, and perhaps are more akin to neonatal myocytes that have a more random arrangement of Ca^{2+} stores. However, as in atrial myocytes, the participation of central RyRs within the neuronal soma depends on the Ca^{2+} content of the intracellular stores or co-stimulation with agonists that sensitise internal Ca^{2+} release (Shmigol et al., 1995).

Conclusion

Atrial myocytes use spatially graded Ca^{2+} signals to control their contractile function. The Ca^{2+} response that occurs following depolarisation of an atrial myocyte depends on the interplay of multiple processes that serve to increase or reduce cytosolic Ca^{2+} levels. The channels, pumps and transporters that impinge on atrial Ca^{2+} signalling are expressed in regular patterns, so that the Ca^{2+} signals in these cells are stereotypic. Depolarisation triggers Ca^{2+} signals that occur at the periphery of the cells. The success or failure of this subsarcolemmal Ca^{2+} signal to penetrate into the centre of the cells depends substantially on the sensitivity of the non-junctional RyRs and their ability to perceive a triggering signal from behind a mitochondrial and/or SERCA firewall. Positive inotropic stimuli enhance the centripetal propagation of the Ca^{2+} signal, using mechanisms that enhance CICR. Negative inotropes (e.g. acetylcholine and adenosine), by contrast, would be expected to reduce the centripetal propagation of the Ca^{2+} signals and sharpen the subsarcolemmal ring of Ca^{2+}. There is a roughly linear relationship between the inward movement of Ca^{2+} and atrial myocyte shortening. The extent of centripetal propagation of the Ca^{2+} signal therefore provides an analogue control over cell contraction. The spatial continuum of Ca^{2+} signalling from peripherally localised to global responses provides a mechanism by which atrial myocytes can precisely regulate their contribution to blood pumping in the heart. Owing to the paucity of T-tubules in myocytes outside the ventricles, the atrial EC coupling model is the most commonly encountered paradigm in mammalian cardiac tissues. It is the sole mechanism underlying activation of non-mammalian cardiac myocytes.

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References

Alpert, J. S., Petersen, P. and Godtfredsen, J. (1988). Atrial fibrillation: natural history, complications, and management. *Annu. Rev. Med.* **39**, 41-52.

Ayettey, A. S. and Navaratnam, V. (1978). The T-tubule system in the specialized and general myocardium of the rat. *J. Anat.* **127**, 125-140.

Balijepalli, R. C., Lokuta, A. J., Maertz, N. A., Buck, J. M., Haworth, R. A., Valdivia, H. H. and Kamp, T. J. (2003). Depletion of T-tubules and specific subcellular changes in sarcolemmal proteins in tachycardia-induced heart failure. *Cardiovasc. Res.* **59**, 67-77.

Bassani, J. W., Yuan, W. and Bers, D. M. (1995). Fractional SR Ca release is regulated by trigger Ca and SR Ca content in cardiac myocytes. *Am. J. Physiol.* **268**, 1313-1319.

Beard, N. A., Casarotto, M. G., Wei, L., Varsanyi, M., Laver, D. R. and Dulhunty, A. F. (2005). Regulation of ryanodine receptors by calsequestrin: effect of high luminal Ca^{2+} and phosphorylation. *Biophys. J.* **88**, 3444-3454.

Berlin, J. R. (1995). Spatiotemporal changes of Ca^{2+} during electrically evoked contractions in atrial and ventricular cells. *Am. J. Physiol.* **269**, 1165-1170.

Berridge, M. J., Bootman, M. D. and Lipp, P. (1998). Calcium – a life and death signal. *Nature* **395**, 645-648.

Berridge, M. J., Lipp, P. and Bootman, M. D. (2000). The versatility and universality of calcium signalling. *Nat. Rev. Mol. Cell. Biol.* **1**, 11-21.

Berridge, M. J., Bootman, M. D. and Roderick, H. L. (2003). Calcium signalling: dynamics, homeostasis and remodelling. *Nat. Rev. Mol. Cell. Biol.* **4**, 517-529.

Bers, D. M. (2002). Cardiac excitation-contraction coupling. *Nature* **415**, 198-205.

Bers, D. M. (2004). Macromolecular complexes regulating cardiac ryanodine receptor function. *J. Mol. Cell. Cardiol.* **37**, 417-429.

Bootman, M., Niggli, E., Berridge, M. and Lipp, P. (1997). Imaging the hierarchical Ca^{2+} signalling system in HeLa cells. *J. Physiol.* **499**, 307-314.

Bootman, M. D., Collins, T. J., Peppiatt, C. M., Prothero, L. S., MacKenzie, L., De Smet, P., Travers, M., Tovey, S. C., Seo, J. T., Berridge, M. J. et al. (2001a). Calcium signalling–an overview. *Semin. Cell. Dev. Biol.* **12**, 3-10.

Bootman, M. D., Lipp, P. and Berridge, M. J. (2001b). The organisation and functions of local Ca^{2+} signals. *J. Cell Sci.* **114**, 2213-2222.

Bootman, M., Berridge, M. and Roderick, H. (2002). Calcium signalling: more messengers, more channels, more complexity. *Curr. Biol.* **12**, 563.

Boyden, P. A., Pu, J., Pinto, J. and Keurs, H. E. (2000). Ca(2+) transients and Ca(2+) waves in purkinje cells: role in action potential initiation. *Circ. Res.* **86**, 448-455.

Brette, F. and Orchard, C. (2003). T-tubule function in mammalian cardiac myocytes. *Circ. Res.* **92**, 1182-1192.

Brette, F., Rodriguez, P., Komukai, K., Colyer, J. and Orchard, C. H. (2004). beta-adrenergic stimulation restores the Ca transient of ventricular myocytes lacking t-tubules. *J. Mol. Cell. Cardiol.* **36**, 265-275.

Brette, F., Despa, S., Bers, D. M. and Orchard, C. H. (2005). Spatiotemporal characteristics of SR Ca(2+) uptake and release in detubulated rat ventricular myocytes. *J. Mol. Cell. Cardiol.* **39**, 804-812.

Cannell, M. B., Cheng, H. and Lederer, W. J. (1995). The control of calcium release in heart muscle. *Science* **268**, 1045-1049.

Carl, S. L., Felix, K., Caswell, A. H., Brandt, N. R., Ball, W. J., Jr, Vaghy, P. L., Meissner, G. and Ferguson, D. G. (1995). Immunolocalization of sarcolemmal dihydropyridine receptor and sarcoplasmic reticular triadin and ryanodine receptor in rabbit ventricle and atrium. *J. Cell. Biol.* **129**, 672-682.

Charles, A. (2005). Glial intercellular waves. *Sci. STKE* **2005**, tr19.

Chen, F., Mottino, G., Klitzner, T. S., Philipson, K. D. and Frank, J. S. (1995). Distribution of the Na^{+}/Ca^{2+} exchange protein in developing rabbit myocytes. *Am. J. Physiol.* **268**, 1126-1132.

Chen-Izu, Y., McCulle, S. L., Ward, C. W., Soeller, C., Allen, B. M., Rabang, C., Cannell, M. B., Balke, C. W. and Izu, L. T. (2006). Three-dimensional distribution of ryanodine receptor clusters in cardiac myocytes. *Biophys. J.* **91**, 1-13.

Cheng, H., Lederer, W. J. and Cannell, M. B. (1993). Calcium sparks: elementary events underlying excitation-contraction coupling in heart muscle. *Science* **262**, 740-744.

Cheng, H., Cannell, M. B. and Lederer, W. J. (1995). Partial inhibition of Ca^{2+} current by methoxyverapamil (D600) reveals spatial nonuniformities in $[Ca^{2+}]_i$ during excitation-contraction coupling in cardiac myocytes. *Circ. Res.* **76**, 236-241.

Cleemann, L., Wang, W. and Morad, M. (1998). Two-dimensional confocal images of organization, density, and gating of focal Ca^{2+} release sites in rat cardiac myocytes. *Proc. Natl. Acad. Sci. USA* **95**, 10984-10989.

Coombes, S., Hinch, R. and Timofeeva, Y. (2004). Receptors, sparks and waves in a fire-diffuse-fire framework for calcium release. *Prog. Biophys. Mol. Biol.* **85**, 197-216.

Cordeiro, J. M., Spitzer, K. W., Giles, W. R., Ershler, P. E., Cannell, M. B. and Bridge, J. H. (2001). Location of the initiation site of calcium transients and sparks in rabbit heart Purkinje cells. *J. Physiol.* **531**, 301-314.

Diaz, M. E., Eisner, D. A. and O'Neill, S. C. (2002). Depressed ryanodine receptor activity increases variability and duration of the systolic Ca^{2+} transient in rat ventricular myocytes. *Circ. Res.* **91**, 585-593.

Diaz, M. E., Graham, H. K., O'Neill S, C., Trafford, A. W. and Eisner, D. A. (2005). The control of sarcoplasmic reticulum Ca content in cardiac muscle. *Cell Calcium* **38**, 391-396.

Duchen, M. R. (2000). Mitochondria and calcium: from cell signalling to cell death. *J. Physiol.* **529**, 57-68.

Eisner, D. A. and Sipido, K. R. (2004). Sodium calcium exchange in the heart: necessity or luxury? *Circ. Res.* **95**, 549-551.

Fill, M. and Copello, J. A. (2002). Ryanodine receptor calcium release channels. *Physiol. Rev.* **82**, 893-922.

Frank, K. F., Bolck, B., Erdmann, E. and Schwinger, R. H. (2003). Sarcoplasmic reticulum Ca^{2+}-ATPase modulates cardiac contraction and relaxation. *Cardiovasc. Res.* **57**, 20-27.

Franzini-Armstrong, C., Protasi, F. and Tijskens, P. (2005). The assembly of calcium release units in cardiac muscle. *Ann. NY Acad. Sci.* **1047**, 76-85.

Go, L. O., Moschella, M. C., Watras, J., Handa, K. K., Fyfe, B. S. and Marks, A. R. (1995). Differential regulation of two types of intracellular calcium release channels during end-stage heart failure. *J. Clin. Invest.* **95**, 888-894.

Gomez, A. M., Valdivia, H. H., Cheng, H., Lederer, M. R., Santana, L. F., Cannell, M. B., McCune, S. A., Altschuld, R. A. and Lederer, W. J. (1997). Defective excitation-contraction coupling in experimental cardiac hypertrophy and heart failure. *Science* **276**, 800-806.

Gotoh, T. (1983). Quantitative studies on the ultrastructural differentiation and growth of mammalian cardiac muscle cells. The atria and ventricles of the cat. *Acta Anat. (Basel)* **115**, 168-177.

Guatimosim, S., Dilly, K., Santana, L. F., Saleet Jafri, M., Sobie, E. A. and Lederer, W. J. (2002). Local Ca(2+) signaling and EC coupling in heart: Ca(2+) sparks and the regulation of the [Ca(2+)](i) transient. *J. Mol. Cell. Cardiol.* **34**, 941-950.

Gunter, T. E., Yule, D. I., Gunter, K. K., Eliseev, R. A. and Salter, J. D. (2004). Calcium and mitochondria. *FEBS Lett.* **567**, 96-102.

Gyorke, S., Gyorke, I., Terentyev, D., Viatchenko-Karpinski, S. and Williams, S. C. (2004). Modulation of sarcoplasmic reticulum calcium release by calsequestrin in cardiac myocytes. *Biol. Res.* **37**, 603-607.

Haddock, P. S., Coetzee, W. A., Cho, E., Porter, L., Katoh, H., Bers, D. M., Jafri, M. S. and Artman, M. (1999). Subcellular $[Ca^{2+}]i$ gradients during excitation-contraction coupling in newborn rabbit ventricular myocytes. *Circ. Res.* **85**, 415-427.

Hatem, S. N., Benardeau, A., Rucker-Martin, C., Marty, I., de Chamisso, P., Villaz, M. and Mercadier, J. J. (1997). Different compartments of sarcoplasmic reticulum participate in the excitation-contraction coupling process in human atrial myocytes. *Circ. Res.* **80**, 345-353.

He, J., Conklin, M. W., Foell, J. D., Wolff, M. R., Haworth, R. A., Coronado, R. and Kamp, T. J. (2001). Reduction in density of transverse tubules and L-type Ca(2+) channels in canine tachycardia-induced heart failure. *Cardiovasc. Res.* **49**, 298-307.

Huser, J., Lipsius, S. L. and Blatter, L. A. (1996). Calcium gradients during excitation-contraction coupling in cat atrial myocytes. *J. Physiol.* **494**, 641-651.

Inoue, M. and Bridge, J. H. (2003). Ca^{2+} sparks in rabbit ventricular myocytes evoked by action potentials: involvement of clusters of L-type Ca^{2+} channels. *Circ. Res.* **92**, 532-538.

Isenberg, G., Etter, E. F., Wendt-Gallitelli, M. F., Schiefer, A., Carrington, W. A., Tuft, R. A. and Fay, F. S. (1996). Intrasarcomere $[Ca^{2+}]$ gradients in ventricular myocytes revealed by high speed digital imaging microscopy. *Proc. Natl. Acad. Sci. USA* **93**, 5413-5418.

Izu, L. T., Means, S. A., Shadid, J. N., Chen-Izu, Y. and Balke, C. W. (2006). Interplay of ryanodine receptor distribution and calcium dynamics. *Biophys. J.* **91**, 95-112.

Jones, P. P., Bazzazi, H., Kargacin, G. J. and Colyer, J. (2006). Inhibition of cAMP-dependent protein kinase under conditions occurring in the cardiac dyad during a Ca^{2+} transient. *Biophys. J.* **91**, 433-443.

Jorgensen, A. O., Shen, A. C., Arnold, W., McPherson, P. S. and Campbell, K. P. (1993). The Ca^{2+}-release channel/ryanodine receptor is localized in junctional and corbular sarcoplasmic reticulum in cardiac muscle. *J. Cell. Biol.* **120**, 969-980.

Keizer, J., Smith, G. D., Ponce-Dawson, S. and Pearson, J. E. (1998). Saltatory propagation of Ca^{2+} waves by Ca^{2+} sparks. *Biophys. J.* **75**, 595-600.

Kirk, M. M., Izu, L. T., Chen-Izu, Y., McCulle, S. L., Wier, W. G., Balke, C. W. and Shorofsky, S. R. (2003). Role of the transverse-axial tubule system in generating calcium sparks and calcium transients in rat atrial myocytes. *J. Physiol.* **547**, 441-451.

Kockskamper, J., Sheehan, K. A., Bare, D. J., Lipsius, S. L., Mignery, G. A. and Blatter, L. A. (2001). Activation and propagation of Ca(2+) release during excitation-contraction coupling in atrial myocytes. *Biophys. J.* **81**, 2590-2605.

Koizumi, S., Lipp, P., Berridge, M. J. and Bootman, M. D. (1999). Regulation of ryanodine receptor opening by lumenal Ca(2+) underlies quantal Ca(2+) release in PC12 cells. *J. Biol. Chem.* **274**, 33327-33333.

Landgraf, G., Gellerich, F. N. and Wussling, M. H. (2004). Inhibitors of SERCA and mitochondrial Ca-uniporter decrease velocity of calcium waves in rat cardiomyocytes. *Mol. Cell. Biochem.* **256-257**, 379-386.

Laver, D. R. (2005). Coupled calcium release channels and their regulation by luminal and cytosolic ions. *Eur. Biophys. J.* **34**, 359-368.

Li, X., Zima, A. V., Sheikh, F., Blatter, L. A. and Chen, J. (2005). Endothelin-1-induced arrhythmogenic Ca^{2+} signaling is abolished in atrial myocytes of inositol-1,4,5-trisphosphate(IP3)-receptor type 2-deficient mice. *Circ. Res.* **96**, 1274-1281

Lipp, P., Pott, L., Callewaert, G. and Carmeliet, E. (1990). Simultaneous recording of Indo-1 fluorescence and Na^{+}/Ca^{2+} exchange current reveals two components of Ca^{2+} release from sarcoplasmic reticulum of cardiac atrial myocytes. *FEBS Lett.* **275**, 181-184.

Lipp, P., Huser, J., Pott, L. and Niggli, E. (1996). Spatially non-uniform Ca^{2+} signals induced by the reduction of transverse tubules in citrate-loaded guinea-pig ventricular myocytes in culture. *J. Physiol.* **497**, 589-597.

Lipp, P., Laine, M., Tovey, S. C., Burrell, K. M., Berridge, M. J., Li, W. and Bootman,

M. D. (2000). Functional InsP3 receptors that may modulate excitation-contraction coupling in the heart. *Curr. Biol.* **10**, 939-942.

Lo, H. M., Lin, F. Y., Lin, J. L., Hsu, K. L., Chiang, F. T., Tseng, C. D. and Tseng, Y. Z. (1999). Impaired cardiac performance relating to delayed left atrial activation after atrial compartment operation for chronic atrial fibrillation. *Pacing Clin. Electrophysiol.* **22**, 379-381.

Louch, W. E., Bito, V., Heinzel, F. R., Macianskiene, R., Vanhaecke, J., Flameng, W., Mubagwa, K. and Sipido, K. R. (2004). Reduced synchrony of Ca^{2+} release with loss of T-tubules-a comparison to Ca^{2+} release in human failing cardiomyocytes. *Cardiovasc. Res.* **62**, 63-73.

Luss, I., Boknik, P., Jones, L. R., Kirchhefer, U., Knapp, J., Linck, B., Luss, H., Meissner, A., Muller, F. U., Schmitz, W. et al. (1999). Expression of cardiac calcium regulatory proteins in atrium v ventricle in different species. *J. Mol. Cell. Cardiol.* **31**, 1299-1314.

Mackenzie, L., Bootman, M. D., Berridge, M. J. and Lipp, P. (2001). Predetermined recruitment of calcium release sites underlies excitation-contraction coupling in rat atrial myocytes. *J. Physiol.* **530**, 417-429.

Mackenzie, L., Bootman, M. D., Laine, M., Berridge, M. J., Thuring, J., Holmes, A., Li, W. H. and Lipp, P. (2002). The role of inositol 1,4,5-trisphosphate receptors in Ca(2+) signalling and the generation of arrhythmias in rat atrial myocytes. *J. Physiol.* **541**, 395-409.

Mackenzie, L., Roderick, H. L., Berridge, M. J., Conway, S. J. and Bootman, M. D. (2004a). The spatial pattern of atrial cardiomyocyte calcium signalling modulates contraction. *J. Cell Sci.* **117**, 6327-6337.

Mackenzie, L., Roderick, H. L., Proven, A., Conway, S. J. and Bootman, M. D. (2004b). Inositol 1,4,5-trisphosphate receptors in the heart. *Biol. Res.* **37**, 553-557.

McDonough, S. I., Cseresnyes, Z. and Schneider, M. F. (2000). Origin sites of calcium release and calcium oscillations in frog sympathetic neurons. *J. Neurosci.* **20**, 9059-9070.

Meissner, G. (2004). Molecular regulation of cardiac ryanodine receptor ion channel. *Cell. Calcium* **35**, 621-628.

Neher, E. (1998). Vesicle pools and Ca^{2+} microdomains: new tools for understanding their roles in neurotransmitter release. *Neuron* **20**, 389-399.

Nicod, P., Hillis, L. D., Winniford, M. D. and Firth, B. G. (1986). Importance of the 'atrial kick' in determining the effective mitral valve orifice area in mitral stenosis. *Am. J. Cardiol.* **57**, 403-407.

Paemeleire, K., Martin, P. E., Coleman, S. L., Fogarty, K. E., Carrington, W. A., Leybaert, L., Tuft, R. A., Evans, W. H. and Sanderson, M. J. (2000). Intercellular calcium waves in HeLa cells expressing GFP-labeled connexin 43, 32, or 26. *Mol. Biol. Cell* **11**, 1815-1827.

Parker, I. and Wier, W. G. (1997). Variability in frequency and characteristics of Ca^{2+} sparks at different release sites in rat ventricular myocytes. *J. Physiol.* **505**, 337-344.

Perez, C. G., Copello, J. A., Li, Y., Karko, K. L., Gomez, L., Ramos-Franco, J., Fill, M., Escobar, A. L. and Mejia-Alvarez, R. (2005). Ryanodine receptor function in newborn rat heart. *Am. J. Physiol. Heart Circ. Physiol.* **288**, 2527-2540.

Peskoff, A. and Langer, G. A. (1998). Calcium concentration and movement in the ventricular cardiac cell during an excitation-contraction cycle. *Biophys. J.* **74**, 153-174.

Protasi, F., Sun, X. H. and Franzini-Armstrong, C. (1996). Formation and maturation of the calcium release apparatus in developing and adult avian myocardium. *Dev. Biol.* **173**, 265-278.

Proven, A., Roderick, H. L., Conway, S. J., Berridge, M. J., Horton, J. K., Capper, S. J. and Bootman, M. D. (2006). Inositol 1,4,5-trisphosphate supports the arrhythmogenic action of endothelin-1 on ventricular cardiac myocytes. *J. Cell Sci.* **119**, 3363-3375.

Rizzuto, R. and Pozzan, T. (2006). Microdomains of intracellular Ca^{2+}: molecular determinants and functional consequences. *Physiol. Rev.* **86**, 369-408.

Roderick, H. L., Berridge, M. J. and Bootman, M. D. (2003). Calcium-induced calcium release. *Curr. Biol.* **13**, R425.

Scriven, D. R., Klimek, A., Lee, K. L. and Moore, E. D. (2002). The molecular architecture of calcium microdomains in rat cardiomyocytes. *Ann. NY Acad. Sci.* **976**, 488-499.

Sedarat, F., Xu, L., Moore, E. D. and Tibbits, G. F. (2000). Colocalization of dihydropyridine and ryanodine receptors in neonate rabbit heart using confocal microscopy. *Am. J. Physiol. Heart Circ. Physiol.* **279**, 202-209.

Sheehan, K. A. and Blatter, L. A. (2003). Regulation of junctional and non-junctional sarcoplasmic reticulum calcium release in excitation-contraction coupling in cat atrial myocytes. *J. Physiol.* **546**, 119-135.

Shen, J. X., Wang, S., Song, L. S., Han, T. and Cheng, H. (2004). Polymorphism of Ca^{2+} sparks evoked from in-focus Ca^{2+} release units in cardiac myocytes. *Biophys. J.* **86**, 182-190.

Shiels, H. A. and White, E. (2005). Temporal and spatial properties of cellular Ca^{2+} flux in trout ventricular myocytes. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **288**, 756-766.

Shmigol, A., Verkhratsky, A. and Isenberg, G. (1995). Calcium-induced calcium release in rat sensory neurons. *J. Physiol.* **489**, 627-636.

Soeller, C. and Cannell, M. B. (2002). Estimation of the sarcoplasmic reticulum Ca^{2+} release flux underlying Ca^{2+} sparks. *Biophys. J.* **82**, 2396-2414.

Sommer, J. R. and Jennings, R. B. (1992). *Ultrastructure of cardiac muscle.* New York: Raven Press Ltd.

Song, L. S., Guatimosim, S., Gomez-Viquez, L., Sobie, E. A., Ziman, A., Hartmann, H. and Lederer, W. J. (2005). Calcium biology of the transverse tubules in heart. *Ann. NY Acad. Sci.* **1047**, 99-111.

Song, L. S., Sobie, E. A., McCulle, S., Lederer, W. J., Balke, C. W. and Cheng, H. (2006). Orphaned ryanodine receptors in the failing heart. *Proc. Natl. Acad. Sci. USA* **103**, 4305-4310.

Stuyvers, B. D., Dun, W., Matkovich, S., Sorrentino, V., Boyden, P. A. and ter Keurs, H. E. (2005). Ca^{2+} sparks and waves in canine purkinje cells: a triple layered system of Ca^{2+} activation. *Circ. Res.* **97**, 35-43.

Tanaka, H., Kawanishi, T. and Shigenobu, K. (2003). Optical bioimaging: from living tissue to a single molecule: atrio-ventricular difference in myocardial excitation-contraction coupling – sequential versus simultaneous activation of SR Ca^{2+} release units. *J. Pharmacol. Sci.* **93**, 248-252.

Thomas, A. P., Bird, G. S., Hajnoczky, G., Robb-Gaspers, L. D. and Putney, J. W., Jr (1996). Spatial and temporal aspects of cellular calcium signaling. *FASEB J.* **10**, 1505-1517.

Tidball, J. G., Cederdahl, J. E. and Bers, D. M. (1991). Quantitative analysis of regional variability in the distribution of transverse tubules in rabbit myocardium. *Cell Tissue Res.* **264**, 293-298.

van der Heyden, M. A., Wijnhoven, T. J. and Opthof, T. (2005). Molecular aspects of adrenergic modulation of cardiac L-type Ca^{2+} channels. *Cardiovasc. Res.* **65**, 28-39.

Wolf, P. A., Mitchell, J. B., Baker, C. S., Kannel, W. B. and D'Agostino, R. B. (1998). Impact of atrial fibrillation on mortality, stroke, and medical costs. *Arch. Intern. Med.* **158**, 229-234.

Woo, S. H., Cleemann, L. and Morad, M. (2002). Ca^{2+} current-gated focal and local Ca^{2+} release in rat atrial myocytes: evidence from rapid 2-D confocal imaging. *J. Physiol.* **543**, 439-453.

Woo, S. H., Cleemann, L. and Morad, M. (2003a). Spatiotemporal characteristics of junctional and nonjunctional focal Ca^{2+} release in rat atrial myocytes. *Circ. Res.* **92**, 1-11.

Woo, S. H., Soldatov, N. M. and Morad, M. (2003b). Modulation of Ca^{2+} signalling in rat atrial myocytes: possible role of the alpha1C carboxyl terminal. *J. Physiol.* **552**, 437-447.

Woo, S. H., Cleemann, L. and Morad, M. (2005). Diversity of atrial local Ca^{2+} signalling: evidence from 2-D confocal imaging in Ca^{2+}-buffered rat atrial myocytes. *J. Physiol.* **567**, 905-921.

Woodcock, E. A. and Matkovich, S. J. (2005). Ins(1,4,5)P3 receptors and inositol phosphates in the heart-evolutionary artefacts or active signal transducers? *Pharmacol. Ther.* **107**, 240-251.

Wu, X., Zhang, T., Bossuyt, J., Li, X., McKinsey, T. A., Dedman, J. R., Olson, E. N., Chen, J., Brown, J. H. and Bers, D. M. (2006). Local InsP3-dependent perinuclear Ca^{2+} signaling in cardiac myocyte excitation-transcription coupling. *J. Clin. Invest.* **116**, 675-682.

Yamasaki, Y., Furuya, Y., Araki, K., Matsuura, K., Kobayashi, M. and Ogata, T. (1997). Ultra-high-resolution scanning electron microscopy of the sarcoplasmic reticulum of the rat atrial myocardial cells. *Anat. Rec.* **248**, 70-75.