Calcium signalling microdomains and the t-tubular system in atrial myocytes: potential roles in cardiac disease and arrhythmias

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Abstract

The atria contribute 25% to ventricular stroke volume and are the site of the commonest cardiac arrhythmia, atrial fibrillation (AF). The initiation of contraction in the atria is similar to that in the ventricle involving a systolic rise of intracellular Ca$^{2+}$ concentration ([Ca$^{2+}$]). There are, however, substantial inter-species differences in the way systolic Ca$^{2+}$ is regulated in atrial cells. These differences are a consequence of a well-developed and functionally relevant transverse (t)-tubule network in the atria of large mammals, including humans, and its virtual absence in smaller laboratory species such as the rat. Where T-tubules are absent, the systolic Ca$^{2+}$ transient results from a ‘fire-diffuse-fire’ sequential recruitment of Ca$^{2+}$ release sites from the cell edge to the centre and hence marked spatiotemporal heterogeneity of systolic Ca$^{2+}$. Conversely, the well-developed T-tubule network in large mammals ensures a near synchronous rise of [Ca$^{2+}$]. In addition to synchronizing the systolic rise of [Ca$^{2+}$], the presence of T-tubules in the atria of large mammals, by virtue of localization of the L-type Ca$^{2+}$ channels and Na$^{+}$–Ca$^{2+}$ exchanger antiporters on the T-tubules, may serve to, respectively, accelerate changes in the amplitude of the systolic Ca$^{2+}$ transient during inotropic manoeuvres and lower diastolic [Ca$^{2+}$]. On the other hand, the presence of T-tubules and hence wider cellular distribution of the Na$^{+}$–Ca$^{2+}$ exchanger may predispose the atria of large mammals to Ca$^{2+}$-dependent delayed afterdepolarizations (DADs); this may be a determining factor in why the atria of large mammals spontaneously develop and maintain AF.

Keywords

Atria • Calcium • T-tubule • Heart failure • Atrial fibrillation

1. Introduction

Increasing longevity of the human population presents a demographic timebomb of cardiovascular disease. Several epidemiological studies have demonstrated a dramatic increase in the prevalence of atrial fibrillation (AF) in elderly populations with an incidence <0.5% in people <50 years of age and in excess of 20% in those over 80 years of age. In addition to the direct effects of the arrhythmia or high atrial rate on ventricular stroke volume noted in some studies, there is also strong association between the presence of AF, occurrence of adverse outcomes following cardiac surgery, and overall development of cerebrovascular stroke. However, despite the significant increase in our understanding of the cellular mechanisms that promote and support the maintenance of AF in animal models and human atrial myocytes, there is still a pressing need for improved therapies for preventing AF development as well as termination of pre-existing AF.

Given the central role that Ca$^{2+}$ plays in initiating contraction and controlling the inotropic status of the heart and as a key cause of many types of cardiac arrhythmia, it should be of no surprise that much of our understanding of how atrial function is regulated and the cellular mechanisms of AF has been gleaned from numerous studies investigating atrial cellular Ca$^{2+}$ homoeostasis and has been the subject of several recent reviews. However, as will be reviewed herein, much of this extant understanding of how atrial excitation contraction coupling is regulated and hence potentially perturbed in diseases where AF is prevalent has been based on studies performed in small laboratory species. As will be discussed, recent
data provide compelling evidence for fundamental structural differences between atrial myocytes from smaller laboratory species and larger mammals, including humans. These structural differences, or more specifically, the presence of a well-developed T-tubule network in atrial cells from large mammals, including humans, should profoundly alter our view of atrial Ca\(^{2+}\) homeostasis and the potential relevance regarding its perturbation in a disease setting. We will (i) compare normal Ca\(^{2+}\) homeostasis between atrial and ventricular cells, (ii) describe the role of T-tubules in atrial cells and how they impact on the generation of the systolic Ca\(^{2+}\) transient and inotropic responsiveness of the atria, (iii) consider whether microdomains of elevated [Ca\(^{2+}\)], arise as a consequence of atrial T-tubules and whether this impacts on diastolic [Ca\(^{2+}\)], (iv) discuss the factors that may be important in the formation of T-tubules, and finally, (v) address the potential role of T-tubules in the pathophysiology of AF.

2. Atrial excitation contraction coupling

As in the extensively studied ventricle (for comprehensive review, see Bers\(^{22}\)), contraction in the atria is initiated by the systolic rise of [Ca\(^{2+}\)]. Opening of voltage-gated L-type Ca\(^{2+}\) channels allows a small amount of Ca\(^{2+}\) to enter the cell on the L-type Ca\(^{2+}\) current \(I_{\text{Ca,L}}\), which then triggers the release of a much greater amount of Ca\(^{2+}\) from the intracellular Ca\(^{2+}\) store, the sarcoplasmic reticulum (SR), and gives rise to the systolic Ca\(^{2+}\) transient and contraction. Relaxation, on the other hand, is brought about by resequestration of Ca\(^{2+}\) back in to the SR via the SR Ca\(^{2+}\)-ATPase (SERCA) and Ca\(^{2+}\)-efflux from the cell by, primarily, the Na\(^+\)-Ca\(^{2+}\) exchanger (NCX) and to a lesser extent by the plasmalemmal Ca\(^{2+}\)-ATPase (PMCA)\(^{23,24}\). While the same ‘machinery’ is used to generate the tosic Ca\(^{2+}\) transient in atrial and ventricular cells, there are substantial inter-chamber differences in the way in which the systolic Ca\(^{2+}\) transient is controlled. For example in the rat, atrial cells are smaller and have a reduced Ca\(^{2+}\)-transient amplitude, accelerated rates of [Ca\(^{2+}\)] decay due to enhanced SERCA activity, greater Ca\(^{2+}\) buffering capacity, and elevated SR Ca\(^{2+}\) content content than is observed in ventricular cells.\(^{23}\) Some of these differences, for example, the accelerated rate of relaxation, are largely biochemical in origin; atrial cells have a higher ratio of SERCA to its inhibitory peptide phospholamban.\(^{22,24}\) However, in other cases such as the heterogeneity of the systolic Ca\(^{2+}\) transient in atrial cells, the cause appears to be of a structural nature, given that atrial cells from smaller species either completely lack or possess only a rudimentary T-tubule network (Figure 1A).

2.1 Rudimentary T-tubule network in small laboratory species

As noted above, in some instances in atrial cells from smaller laboratory species, including the rat\(^{28}\) and the mouse,\(^{29}\) a ‘t-tubule’ network is sometimes observed. However, in these smaller species the atrial T-tubule network tends to be architecturally distinct from that present in the ventricle. In ventricular cells, the dominant T-tubule features run perpendicular to the long axis of the cell along each z-line with branching between adjacent tubules.\(^{30-32}\) (see Figures 1A and 2A).

Conversely, in those atrial cells that possess t-tubular structures, the network or transverse axial tubules (TATs) tend to run mainly along the long axis of the cell and appear relatively disorganized compared with ventricular structures (see Figures 1A and 2A).\(^{28,33,34}\)

Where T-tubules are absent in atrial cells, the consequences for the spatial and temporal properties of the systolic Ca\(^{2+}\) transient are predictable with the initial rise of [Ca\(^{2+}\)], on depolarization occurring at the cell periphery and then Ca\(^{2+}\) being released sequentially as a wave of Ca\(^{2+}\)-induced Ca\(^{2+}\) release (CICR) propagating towards the cell centre. This ‘fire-diffuse-fire’ mechanism\(^{35-37}\) produces the characteristic delayed central Ca\(^{2+}\) transient observed as a V- or V’-shaped [Ca\(^{2+}\)], profile in confocal line scan (xt) images (Figure 1).\(^{38-40}\) In addition to the absence of T-tubules, the increased Ca\(^{2+}\) buffering capacity of atrial cells\(^{23,41}\) and the subsarcolemmal Ca\(^{2+}\) ‘diffusion barrier’ of the mitochondria and SERCA pumps.\(^{39}\) As depicted in Figure 1C, can also cause the systolic Ca\(^{2+}\) transient in atrial cells to be restricted to the subsarcolemmal region with no propagation of the Ca\(^{2+}\) transient to the cell centre.\(^{39,41,42}\)

The effect that the TAT network has on the spatial and temporal properties of the systolic Ca\(^{2+}\) transient is, on the other hand, more unpredictable and presumably depends largely on the extent of the network. However, in those studies where TATs have been reported and [Ca\(^{2+}\)], imaged, there is still a marked non-uniformity of the systolic Ca\(^{2+}\) transient with a definite serrated or sawtooth appearance to the leading edge of the Ca\(^{2+}\) transient when imaged using xt line scanning.\(^{28,34}\)

There are at least two additional important factors that will determine the degree of heterogeneity of the systolic rise of [Ca\(^{2+}\)], in atrial cells where a TAT network is present: (i) whether or not functional couplons (L-type Ca\(^{2+}\) channels and apposed RyRs) exist on the TAT network and (ii) whether TAT membranes are depolarized during the atrial action potential. Regarding the potential importance of functional couplons on the TAT network, some data exist suggesting that compared with the situation in ventricular cells where co-localization of L-type Ca\(^{2+}\) channels and RyRs occurs,\(^{43}\) there is only very weak association or co-localization of L-type Ca\(^{2+}\) channels and RyRs in atrial cells where TATs have been noted.\(^{44,44,45}\) This lack of co-localization by definition implies that there will be few functional Ca\(^{2+}\) release units, or couplons, on the TAT network and, therefore, generation of the systolic Ca\(^{2+}\) transient in the centre of these cells is still likely to be mainly reliant on a ‘fire-diffuse-fire’ model for Ca\(^{2+}\) release; however, an \(I_{\text{Ca,L}}\)-dependent rapid component of central Ca\(^{2+}\) release has been reported in some rat atrial myocytes.\(^{41,46}\)

With regard to the second point, conclusive experimental evidence in the atria is lacking for whether TATs are electrically coupled to the surface sarcolemma and therefore depolarize upon arrival of the action potential; conclusive experimental evidence in the atria is lacking. However, at least in the ventricle tight electrical coupling between the surface sarcolemma, transverse (t), and axial (TAT) tubular network occurs.\(^{47}\) However, and potentially of particular relevance to small mammalian atrial cells where the TAT network appears quite disordered, in heart failure where the TAT network of ventricular cells is disrupted, the electrical coupling between the surface sarcolemma, remaining T-tubules, and the TAT network decreases.\(^{47}\)

2.2 Ca\(^{2+}\) signalling macro and microdomains in small mammalian atrial cells

Much of the foregoing discussion has highlighted the rather obvious spatial heterogeneity of the atrial systolic Ca\(^{2+}\) transient in small
species, such as the rat, mouse, and cat, and thus macrodomains of differing Ca\textsuperscript{2+} concentrations. However, many of the early studies also noted that these atrial myocytes also possessed so-called ‘eager sites’, where Ca\textsuperscript{2+} sparks were observed to occur with an increased frequency compared with the general cytosolic volume of the cell.\textsuperscript{28,48,49} Ca\textsuperscript{2+} sparks are the elementary Ca\textsuperscript{2+} release event or building block of the systolic Ca\textsuperscript{2+} transient\textsuperscript{50–52} and produce microdomains of increased \([\text{Ca}^{2+}]_i\). In ventricular cells, Ca\textsuperscript{2+} sparks are predominantly localized to the z-lines along the length of T-tubules supporting the role of the couplon of the L-type Ca\textsuperscript{2+} channel and RyR clusters in their initiation.\textsuperscript{51–53} In rat atrial cells, however, the picture is somewhat different with Ca\textsuperscript{2+} sparks being preferentially located to the cell periphery; again this is consistent with a role for couplons in their initiation, given the apposition of L-type Ca\textsuperscript{2+} channels and RyR clusters around the surface of atrial cells lacking T-tubules.\textsuperscript{38,48,49} However, at least in atrial cells, there may be a second factor that restricts Ca\textsuperscript{2+} sparks to the cell periphery and that is the preferential surface sacrolemmal localization of inositol 1,4,5-trisphosphate receptors (IP\textsubscript{3}R\textsubscript{S}).\textsuperscript{18,54} In this paradigm, many receptor-signalling cascades, e.g. endothelin, angiotensin II, noradrenaline (\(\alpha_1\) agonism), and acetylcholine (muscarnic m\textsubscript{1} and m\textsubscript{3}), lead to the generation of IP\textsubscript{3} and activation of Ca\textsuperscript{2+} release from IP\textsubscript{3} sensitive Ca\textsuperscript{2+} stores and modulate systolic Ca\textsuperscript{2+} possibly via the released Ca\textsuperscript{2+} augmenting RyR-dependent Ca\textsuperscript{2+} release (reviewed in\textsuperscript{55}). Finally, a key question arising from these studies and the differing spatial distribution of Ca\textsuperscript{2+} sparks between rat atrial and ventricular cells is whether or not the Ca\textsuperscript{2+} sparks influence diastolic \([\text{Ca}^{2+}]_i\) or the susceptibility of atrial cells to Ca\textsuperscript{2+}-dependent arrhythmias (DADs). This point will be discussed in Sections 3.3 and 3.4, where the role of T-tubules in atrial cells of large mammalian species is considered.

3. Transverse (t)-tubules in atrial cells from large mammals

An early study measuring \([\text{Ca}^{2+}]_i\) in freshly isolated human atrial cells stated that the rising phase of the systolic Ca\textsuperscript{2+} transient, in the majority of cells, had two distinct components including a fast initial rate of rise of \([\text{Ca}^{2+}]_i\) followed by a slow increase in \([\text{Ca}^{2+}]_i\), to a plateau.\textsuperscript{56} Such an observation, two components to the rising phase of the systolic Ca\textsuperscript{2+} transient, is consistent with several previous studies in smaller species where a T-tubule network is ostensibly absent. However, this

![Figure 1](https://academic.oup.com/cardiovascres/article-abstract/98/2/192/277317/194)
initial concept that (most) human atrial cells lack a well-developed T-tubule network has recently been revised by several studies demonstrating that atrial cells from large mammals (including humans) possess a well-developed and functional T-tubule network.9,20,21

In the following sections, we will review how this T-tubule network impacts on the systolic Ca$^{2+}$ transient and the consequences of its remodelling in cardiac pathological states. However, the mere presence of this T-tubule network fundamentally alters our understanding of how atrial excitation contraction coupling is controlled and may be a significant factor predisposing large mammals to spontaneously develop, and sustain, AF. As such, careful consideration should be given to the animal model used for future mechanistic and therapeutic studies of AF.

3.1 A functional T-tubule network is present in the atria of large mammals

Dibb et al.21 and Lenaerts et al.9 both reported in 2009 that atrial myocytes isolated from the sheep possess a well-developed T-tubule network that was, respectively, disrupted in heart failure and AF. Figure 2 provides the important observation that atrial T-tubules are not restricted to the sheep and that an extensive T-tubule network in several large mammalian species including, humans.20 However, the extent of the T-tubule network across these species, and indeed within the human atrium (Figure 2E), does exhibit some variability. Such variability may be due to regional differences (left vs. right atria; appendage vs. free wall, etc9,20,21) or more simply arise as a consequence of heterogeneity in cell size with narrower cells having fewer T-tubules than wider cells.20 However, the prospect of inter-atrial differences in T-tubule density (left vs. right; compare Dibb et al.21 and Lenaerts et al.9) does raise the interesting possibility that differences in T-tubule distribution may be, through an impact on ion channel distribution and cellular Ca$^{2+}$ homoeostasis (see section 5), causally linked to the development of AF which is more commonly initiated in the left than right atria57.

In view of the presence of an extensive T-tubular network in larger mammals, albeit to slightly differing extents between atrial regions and
species, two important questions are (i) is this t-tubular network functional? and (ii) does the T-tubule network impact on the control of the systolic Ca\(^{2+}\) transient? As illustrated in Figure 3, the T-tubules found in sheep atrial cells lead to the generation of a Ca\(^{2+}\) transient that rises simultaneously at the cell edge and cell centre; this is in stark contrast to that observed in smaller species where the T-tubule network is essentially absent (Figure 1B). That the systolic Ca\(^{2+}\) transient at the cell edge and centre has the same temporal profile has two important implications: it suggests that (i) functional couplons are present along the T-tubule network and (ii) the T-tubules are depolarized at the same time as the cell surface; the anatomical arrangement of atrial cells where T-tubules are present is depicted schematically in Figure 3D. In summary, the presence of couplons at the cell surface and throughout the depth of the atrial myocyte along the z-line/t-tubule axis leads to uniform triggering of Ca\(^{2+}\) release throughout the cell.

### 3.2 Atrial T-tubules and regulation of systolic Ca\(^{2+}\)

The preceding discussion identified the presence of a functionally relevant T-tubule network in the atria of large mammals, including humans. This, therefore, necessitates a revision of the concepts of the processes initiating and hence regulating the atrial systolic Ca\(^{2+}\) transient. However, considerable additional work is required to establish how important this atrial T-tubule network is for altering or

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**Figure 3** Atrial T-tubules are associated with triggered Ca\(^{2+}\) release. (A) X–y images taken at 17 ms intervals showing [Ca\(^{2+}\)]\(_i\), measured using Fluo-5F during an external stimulus. (B) Di-4-ANEPPS staining of surface and T-tubule structures (i) and [Ca\(^{2+}\)]\(_i\), from the 5th frame in A (ii). The arrows in image (ii) are in the same position as in image (i). (iii) The spatial profile of [Ca\(^{2+}\)]\(_i\) along a horizontal line given by the white arrow in (ii). (C) Ca\(^{2+}\) transients recorded from the cell periphery (a) and centre (b). Measurements were taken from rectangular areas at the horizontal lines in (Bii) extending the whole height of the image. Modified from Dibb et al\(^{21}\). (D) Schematic representation of Ca\(^{2+}\) handling and contractile machinery in an atrial myocyte with T-tubules. Cellular components are as shown in Figure 1.
modulating the inotropic response of the atria in large mammals. For instance, in atrial cells where T-tubules are absent such as in the rat, the systolic Ca\(^{2+}\) transient can be initiated only at the cell periphery and may be restricted to this site for reasons discussed above. Under these circumstances, due to the lack of a T-tubule network, any G-protein-coupled receptor (GPCR) involved in inotropism, e.g., \(\beta\)-adrenergic (\(\beta\)-AR) receptors, can also be present only at the cell surface. Thus, the effect that the activation of any of these receptors has on the systolic Ca\(^{2+}\) transient will depend not only on the local effects on Ca\(^{2+}\) release, e.g., via increased \(I_{Ca}\), initiating greater centripetal Ca\(^{2+}\) wave propagation, but also on the 'reach' of the intracellular signalling cascade. Based on data obtained in ventricular cells, \(\beta\)-AR signalling and target phosphorylation are spatially restricted, whereas \(\beta\)-AR-dependent phosphorylation events are diffuse.\(^{58}\)

Thus, where T-tubules are present in the atria, the inotropic response to \(\beta_1\) or \(\beta_2\)-AR stimulation could be markedly different from that in cells where T-tubules are absent, and indeed when they (T-tubules) remodel during cardiac diseases.\(^{9,21}\) As in the ventricle, T-tubule remodelling in the atria in cardiac disease states may have important implications for the genesis of arrhythmias via initiation of Ca\(^{2+}\)-dependent delayed afterdepolarizations (DADs) during, for example, \(\beta_2\)-AR stimulation.\(^{59}\) Similar considerations regarding the presence of T-tubules and the distribution and reach of the intracellular signalling cascades also need to be applied to other GPCRs (endothelin, angiotensin, etc) that are known to modulate atrial Ca\(^{2+}\) homeoestasis and have been implicated in the initiation of AF (for reviews, see\(^{9,60,61}\)).

### 3.3 Atrial T-tubules, [Ca\(^{2+}\)], microdomains, diastolic [Ca\(^{2+}\)], and arrhythmias

Another unresolved question regarding atrial T-tubules involves their importance in determining where Ca\(^{2+}\) sparks occur and hence a potential impact control of diastolic [Ca\(^{2+}\)], and the initiation of DADs. As discussed in Section 2.2, where T-tubules are absent in atrial myocytes of rodents, etc; Ca\(^{2+}\) sparks occur preferentially around the cell periphery. In contrast, based on findings in ventricular cells, there is good reason to believe that in atrial cells possessing a well-developed T-tubule system, Ca\(^{2+}\) sparks are at least equally, or more, likely to occur in the cell interior/along z-lines; indeed such a predilection for Ca\(^{2+}\) sparks occurring in the cell interior has been noted in some human atrial myocyte studies.\(^{62,63}\) The premise of this prediction is based on the existence of functional couplings along the length of the T-tubule, a well-organized z-line distribution of RyRs\(^{62,63}\) (schematically summarized in Figure 3D), both of which would facilitate Ca\(^{2+}\) sparks with z-line localization.

Germane to Ca\(^{2+}\) sparks occurring throughout the cytoplasm in atrial cells possessing a T-tubule network are the questions of whether or not these microdomains of elevated [Ca\(^{2+}\)], influence diastolic [Ca\(^{2+}\)], or are pathologically relevant. Considering the issue of a role in regulating diastolic [Ca\(^{2+}\)], first, the initial assumption would be that Ca\(^{2+}\) sparks occurring throughout the cell would elevate [Ca\(^{2+}\)]; and, therefore, impair diastolic function. However, a counter argument is that the coupling, in addition to consisting of the apposed L-type Ca\(^{2+}\) channel and RyR, also incorporates closely located NCX antiporters.\(^{64}\) Under these circumstances NCX, by virtue of proximity, has preferential access to the Ca\(^{2+}\) released from the SR during a Ca\(^{2+}\) spark and, therefore, Ca\(^{2+}\) sparks can serve to lower diastolic [Ca\(^{2+}\)], as some of the Ca\(^{2+}\) released during the spark is removed from the cell by NCX with the remainder being re-sequestered by the SR. Indeed, paradigms where preferential access to the Ca\(^{2+}\) released during a Ca\(^{2+}\) spark serves to modulate distinct aspects of cellular function are well characterized in smooth muscle where Ca\(^{2+}\) sparks activate BKCa channels leading to membrane hyperpolarization and relaxation.\(^{65,66}\)

However, due to the electrogenicity of NCX, the role of facilitating Ca\(^{2+}\) efflux from the cell and lowering diastolic [Ca\(^{2+}\)], in cardiac muscle comes at the expense of an inward depolarizing current and is thus also pro-arrhythmic.\(^{67}\) As such, a careful balance must be achieved to allow the beneficial effects of lowering diastolic [Ca\(^{2+}\)], and Ca\(^{2+}\) efflux from the cell without initiating arrhythmia producing DADs.

In addition to the putative involvement of Ca\(^{2+}\) sparks in regulating diastolic [Ca\(^{2+}\)], discussed above, much has been made in recent years regarding the arrhythmogenic role that Ca\(^{2+}\) sparks, or Ca\(^{2+}\) leak from the SR, play in diverse disease settings such as heart failure\(^{68-71}\) and in the context of this review, AF.\(^{11,13,16,72}\) The basic premise in these studies is that during the disease process the RyR becomes excessively phosphorylated, either by PKA or by Ca\(^{2+}\)-calmodulin kinase-dependent mechanisms, which in turn increases the open probability (\(p_o\)) of the RyR and promotes leak of Ca\(^{2+}\) from the SR in the form of Ca\(^{2+}\) sparks or Ca\(^{2+}\) waves. Subsequent Ca\(^{2+}\) efflux from the cell by NCX leads to depolarization and arrhythmia initiation and maintenance. Of course, in AF, additional electrophysiological remodelling occurs including action potential duration (APD) shortening and reduction of the effective refractory period (ERP)\(^{71}\) and will play a role in perpetuating rotors of excitability.\(^{74,75}\) However, this hypothesis, as a pathological mechanism, requires at least two important notes of caution. First, if increased RyR \(p_o\) and hence Ca\(^{2+}\) leak were the only change to occur, this would lower the SR Ca\(^{2+}\) content and the leak, and thus arrhythmogenic Ca\(^{2+}\) waves, would cease\(^{76,77}\); thus, there must also be a mechanism under these circumstances to maintain the SR Ca\(^{2+}\) content. The second relevant consideration concerns the localization of DAD inducing NCX antiporters and T-tubule remodelling that occurs in the atria in heart failure\(^{21}\) and AF.\(^{73}\) At least in the canine atria where immunolocalization studies have been performed, NCX is concentrated along T-tubules.\(^{28}\) Thus loss of T-tubules would remove a key component of the electrophysiological apparatus required for DAD formation; although increased surface sarcolemmal expression of NCX and/or altered Ca\(^{2+}\) dependence of the exchanger could compensate for the loss of T-tubules. Nevertheless, in the absence of T-tubules any spontaneous Ca\(^{2+}\) sparks or waves occurring in the cell interior would not activate NCX and thus depolarization.

### 3.4 Atrial T-tubules, L-type Ca\(^{2+}\) channels, and inotropy

The L-type Ca\(^{2+}\) current has two roles in excitation contraction coupling; first, a triggering role as Ca\(^{2+}\) entering via \(I_{Ca-L}\) initiates Ca\(^{2+}\) release from the SR and, secondly, the Ca\(^{2+}\) entering the cell via \(I_{Ca-L}\) has a Ca\(^{2+}\) loading role and maintains the SR Ca\(^{2+}\) content.\(^{79,80}\) While direct comparative studies are essentially absent, available data do not support differences in \(I_{Ca-L}\) density (peak inward current normalized to cell capacitance) between atrial and ventricular cells\(^{23,81}\) or between species where atrial T-tubules are present\(^{9,13,17,82}\) or absent.\(^{23,81}\) Nevertheless, we have previously discussed that the presence of L-type Ca\(^{2+}\) channels along T-tubules throughout the depth of the cell has important consequences for
systolic Ca\(^{2+}\) and synchronization of [Ca\(^{2+}\)]\(_i\) (Figures 1, 3, and 4). Additionally, it could be argued that the wider cellular distribution of L-type Ca\(^{2+}\) channels also has an unexpected benefit in the form of accelerating the change in [Ca\(^{2+}\)]\(_i\), and thus contraction during inotropic manoeuvres such as β-AR stimulation.

The reason for suggesting potential involvement of T-tubules in regulating the rapidity of the inotropic response comes from two experimental studies, where changes in Ca\(^{2+}\) transient amplitude in response to alterations in \(I_{\text{Ca-L}}(\left[\text{Ca}^{2+}\right]_o)\) are monitored in rat atrial39 and ventricular83 cells, i.e. in cells, respectively, lacking and possessing T-tubules. In ventricular cells changes in \(I_{\text{Ca-L}}\) immediately produce a steady-state effect on Ca\(^{2+}\)-transient amplitude, whereas in atrial cells the lack of T-tubules is associated with an increase in Ca\(^{2+}\)-transient amplitude requiring many beats to reach a new steady-state level. Similar gradually developing effects on systolic Ca\(^{2+}\) have also been predicted in simulation studies where \(I_{\text{Ca-L}}\) is altered in ‘atrial’ cells.94 The question, therefore, is why might T-tubules accelerate the inotropic response in atrial cells?

Unfortunately, to date, experimental data from suitable species (with T-tubules in their atria) is lacking, and therefore, providing a definitive mechanistic answer remains elusive. However, in an attempt to address this question we consider β-AR stimulation, where the major cause of the inotropic response in an increase in the SR Ca\(^{2+}\) content,85,86 which in turn occurs due to stimulation of SERCA activity. For simplicity, we ignore changes in \(I_{\text{Ca-L}}\) during β-AR stimulation. If we assume that (i) during steady-state conditions the amount of Ca\(^{2+}\) entering the cell on \(I_{\text{Ca-L}}\) is removed from the cell by NCX and (ii) Ca\(^{2+}\) transient amplitude determines Ca\(^{2+}\) efflux on the exchanger,80,83 then to increase SR Ca\(^{2+}\) content requires greater Ca\(^{2+}\) influx on \(I_{\text{Ca-L}}\) than efflux via NCX.80,87 As SERCA activity

**Figure 4** The effect of junctophilin-2 knockdown on the function of T-tubule–SR junctions. Ca\(^{2+}\) spike images (A) and time course (B) elicited by depolarization in a typical control (left) and junctophilin 2 shRNA-treated cells (right). Note the more uniform timing (vertical alignment) of the changes in [Ca\(^{2+}\)], (Ca\(^{2+}\) spikes) in depolarization in the control cell and delayed rising times of Ca\(^{2+}\) spikes in the shRNA knockdown cell. In (B) subsections (a–f) correspond to the vertical location of the Ca\(^{2+}\) spikes used for subsequent time course analysis. Junctophilin -2 knockdown results in a decrease in Ca\(^{2+}\) spike amplitude (C) and decrease in Ca\(^{2+}\) spike synchronicity (D) as evidenced by the increased likelihood of spike failure. Adapted from Wu et al.90
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increases during β-AR stimulation the Ca\(^{2+}\) transient decays more quickly, which reduces Ca\(^{2+}\) efflux from the cell and increases the SR Ca\(^{2+}\) content and thus the amplitude of the subsequent Ca\(^{2+}\) transient. Under these conditions, the SR Ca\(^{2+}\) content and Ca\(^{2+}\)-transient amplitude continue to increase until the amplitude of the systolic Ca\(^{2+}\) transient is such that Ca\(^{2+}\) influx on I\(_{Ca-L}\) and efflux on NCX are once again in balance. Given this mechanism for increasing systolic Ca\(^{2+}\) during β-AR stimulation, the presence of L-type Ca\(^{2+}\) channels throughout the entire cell volume could ensure that the SR Ca\(^{2+}\) content also increases throughout the cell rapidly and therefore accelerates the inotropic response. Conversely, where T-tubules are missing and systolic Ca\(^{2+}\) release, under basal conditions, is normally spatially restricted to the cell periphery, there is still a role for increasing the SR Ca\(^{2+}\) content during β-AR stimulation. However, this increase in the SR Ca\(^{2+}\) content facilitates propagation of the initial peripheral rise of [Ca\(^{2+}\)]\(_{i}\) to the cell centre.\(^{39,88}\) Therefore, the restriction of Ca\(^{2+}\) entry to the cell periphery will initially load the subsarcolemmal junctional SR with Ca\(^{2+}\) which, as the Ca\(^{2+}\) transient increases, will lead to loading of adjacent non-junctional SR with Ca\(^{2+}\) such that over several beats Ca\(^{2+}\) release occurs throughout the cell via Ca\(^{2+}\) wave propagation (a regenerative fire-diffuse-fire process). A simple mathematical model illustrating how I\(_{Ca-L}\) is important in leading to an increase in the SR Ca\(^{2+}\) content and thus positive inotropy has recently been described.\(^{89}\)

Thus in summary, atrial T-tubules not only increase the spatial homogeneity of the systolic Ca\(^{2+}\) transient, but may also serve to accelerate inotropic responses where increase of the SR Ca\(^{2+}\) content is a key cellular mechanism. The key factor by which T-tubules serve to synchronize systolic [Ca\(^{2+}\)]\(_{i}\) and potentially accelerate inotropic responses is through the presence of L-type Ca\(^{2+}\) channels and junctional SR (couplons) along the T-tubule. However, further experimental work is required to determine whether this hypothetical effect of I\(_{Ca-L}\) on facilitating changes of the SR Ca\(^{2+}\) content in cells containing and lacking T-tubules occurs.

4. Regulators of T-tubule formation

As already discussed, there is considerable inter-species and inter-chamber variability in the extent of the T-tubule network in the atria. Additionally, we have also alluded to considerable atrial T-tubule remodelling occurring during diverse cardiac disease states.\(^{2,21}\) Therefore, an important, and currently largely unresolved, issue is what controls T-tubule formation and whether these ‘controlling’ factor or factors offer potential as future therapeutic targets to correct Ca\(^{2+}\) signalling abnormalities in the atria in AF or heart failure. Given the paucity of studies investigating the mechanisms of T-tubule formation in the cardiac muscle,\(^{90–94}\) we will only provide an overview of the candidate proteins involved in T-tubule formation and their potential role in disease states where T-tubule disorganization or loss is known to occur.

Perhaps it is not surprising given the localization of T-tubules (generally) to the z-line that proteins thought to anchor partner proteins to the z-disc or cytoskeleton, such as junctophilin-2, amphiphysin-II, and telothionin, have been implicated in the formation of T-tubules in cardiac and skeletal muscle and trafficking of ion channels to the T-tubule membrane.\(^{90,93,95–98}\) Alterations in the expression of these proteins are observed in disease states where T-tubules are lost or become disorganized\(^{90,95,99,100}\) are consistent with a role of these putative T-tubule controlling proteins. However, a limited number of studies using gene knockdown approaches have recently demonstrated causal links between junctophilin-2,\(^{90,100}\) amphiphysin-II,\(^{91}\) and T-tubule maintenance with loss of T-tubules occurring on gene silencing and subsequent perturbation of the systolic Ca\(^{2+}\) transient (Figure 4).

Given the diversity of proteins and the complexity of protein–protein interactions at the z-disc,\(^{101}\) it would not be surprising if, in the future, the list of candidate proteins involved in T-tubule formation, organization, and maintenance increased considerably or additive roles were found for combinations of proteins. In addition to the proteins linked to T-tubule formation directly and the control of their mRNA expression by micro-RNAs (e.g. junctophilin-2 and Mir-29),\(^{92}\) consideration also needs to be given to second messengers, e.g. phosphatidylinositol 4,5-biphosphate\(^{102}\) and intracellular kinases, e.g. phosphoinositide 3-kinases (PI3Ks).\(^{94}\) Although members of the same signalling cascade, PI3P\(_2\) and PI3K have different potential roles in T-tubule formation. PI3K is implicated in causing conformational changes in membrane structure and thus targeting of amphiphysin-II, whereas PI3K is argued to target proteins such as the L-type Ca\(^{2+}\) channel to the T-tubule and thus maintain T-tubule integrity.

Clearly, therefore, our understanding of what controls T-tubule formation, organization, and ultimately turnover and loss is very much in its infancy. However, T-tubules are exceptionally plastic structures and can ‘recover’ following cessation of the underlying cardiac insult, e.g. mechanical unloading of the heart restores T-tubule density and normalizes Ca\(^{2+}\) transient properties.\(^{103}\) As such there is cause to be optimistic that targeting T-tubule formation and turnover offers a genuine therapeutic target and novel approach to treating cardiac dysfunction that arises as a consequence of T-tubule loss in various disease states.

5. Remodelling of T-tubules in heart failure and atrial fibrillation; consequences for the systolic Ca\(^{2+}\) transient

A number of studies in ventricular myocytes have shown that T-tubules are plastic structures and are dynamically remodelled during various disease processes.\(^{7,103–105}\) In the ventricle, the extent of T-tubule loss and remodelling, however, is relatively mild compared with that which has recently been described in sheep atrial cells following either heart failure\(^{21}\) or AF.\(^{9}\) In the case of heart failure (Figure 5), T-tubule loss in atrial cells is almost complete and the consequences of this T-tubule loss on the systolic Ca\(^{2+}\) transient are dramatic with the Ca\(^{2+}\) transient restricted to the cell periphery.\(^{21}\) In AF on the other hand, the extent of atrial cell T-tubule loss and disorganization is less than occurs in heart failure and, therefore, it is not surprising that rather than being spatially restricted to the cell edges, the systolic rise of Ca\(^{2+}\) occurs more diffusely throughout the cell. Importantly in AF, however, the systolic rise of Ca\(^{2+}\) is considerably less uniform than occurs in control cells and the number of sites where Ca\(^{2+}\) release is delayed greatly increased.\(^{9}\)

5.1 Transverse T-tubule remodelling: is it a cause or consequence of atrial pathology and arrhythmias?

The previous section details the loss of T-tubules in atrial cells in AF and heart failure, where AF is a common finding; however, the key
An unresolved question is whether T-tubules play a role in the development of AF? The recent observation that T-tubules are prevalent in the atria of large mammals\textsuperscript{9,20,21} and as previously established, essentially absent in the atria of small mammals is potentially of fundamental importance in the pathophysiology, and as a mechanism, of AF. That this is the case arises because AF develops spontaneously in these larger species because the size of their atria is sufficiently large there is enough tissue to sustain fibrillatory rotors, i.e. the atria are larger than the wavelength of the rotor.\textsuperscript{106} Conversely, in smaller species where T-tubules are absent, e.g., the mouse, the atria are generally held to be too small to sustain fibrillatory rotors. Given the difference in T-tubule density between atrial cells between the mouse and, for example, sheep, and the ability of the latter but not the former to develop AF this does raise the intriguing possibility that it is the presence of T-tubules that actually predisposes the atria of larger mammals to AF. However, a counter argument is that the atria of large mammals have bigger atrial cells\textsuperscript{20} and as we have already discussed, the size of the atria is important in accommodating fibrillatory rotors and larger atrial cells have greater T-tubule density.\textsuperscript{20} Thus, T-tubules, in this instance, may potentially be nothing more than an innocent bystander present simply because the atrial cells are larger and T-tubules are required to increase the size of the systolic Ca\textsuperscript{2+} transient.

An additional argument against an involvement of T-tubules in the susceptibility to AF is that in heart failure T-tubules completely disappear\textsuperscript{21} (Figure 5A) and, at least in canine models of heart failure, where a similar T-tubule depletion is to be expected given the presence of T-tubules in control canine atrial cells\textsuperscript{78,107} and the same tachypacing method of inducing heart failure,\textsuperscript{108,109} the duration of pacing-induced AF is greatly increased in heart failure.\textsuperscript{108,109} However, in addition to the loss of T-tubules, remodelling of the atrial extracellular matrix (fibrosis) and ion channel expression also occurs in heart failure and is likely a key factor in AF induction (reviewed in\textsuperscript{74,110}), and in this case, the susceptibility to AF would be independent of the presence or absence of T-tubules.

Conversely, and possibly a more likely scenario, atrial T-tubules and their remodelling in various disease states could have two separate roles in the pathogenesis of AF. First, the presence of T-tubules and distribution of NCX on T-tubule membranes\textsuperscript{78} could increase the likelihood of Ca\textsuperscript{2+}-dependent DADs, which could act as the initiators of AF. Then, again because ion channel subunits may be concentrated on the T-tubules as T-tubules remodel and are lost with the development of AF, this may be a major factor determining the decrease in $I_{\text{Ca,L}}$, APD and thus ERP of atrial tissue and thus serve to facilitate excitatory wave front re-entry and perpetuation of AF as the wavelength of the fibrillatory rotor is reduced.

Given the above arguments, the precise role of T-tubules in the initiation and subsequent maintenance phases of AF is far from clear or easily predicted. As a consequence of these uncertainties, considerable additional experimental work will be required to settle this issue. However, a key factor that should be borne in mind is that large mammals, including humans, have a well-developed atrial T-tubule network and these large mammals can spontaneously develop AF. As such, when designing experiments to investigate the pathophysiology of AF careful consideration should be given to the choice of animal model and the hypothesis being examined, i.e. the mere presence of T-tubules might be relevant to AF susceptibility, but the maintenance of AF may be more dependent on the absence of T-tubules or a reduction in their density.

Figure 5 Loss of atrial T-tubules in disease. (A–C) Di-4 or di-8-ANEPPS staining of atrial myocytes isolated from a sheep model of heart failure (A), a sheep model of atrial fibrillation (B), and a canine model of 1 week right atrial tachypacing (C) compared with control atrial myocytes, modified from refs.\textsuperscript{9,21,117}
5.2 Other sites of pathology that may lead to atrial fibrillation

The foregoing review has focused on a putative role for atrial myocyte structural and Ca\(^{2+}\) homeostatic remodelling as a causative factor in the genesis of AF. However, there is well-documented evidence that ‘non-atrial’ regions of the atrial chambers such as the pulmonary vein myocardium may be important in the genesis of AF. The pulmonary vein myocardium appears to have a distinct ontogeny to both the atria and pulmonary vein per se, and interestingly, genetic polymorphisms on chromosomes 4q25 surrounding one of the transcription factors responsible for early pulmonary vein myocardium formation, Ptx2c, are strongly associated with future AF development. Moreover, compared with atrial cells, myocytes isolated from the pulmonary vein sleeve exhibit subtle differences in intracellular Ca\(^{2+}\) regulation that may make them more prone to spontaneous firing and act as a focus for AF initiation. Nevertheless, there is substantial incontrovertible evidence linking changes in atrial myocyte structure and cellular Ca\(^{2+}\) homeostasis to the initiation and maintenance of AF, and the important question is if these changes are prevented or reversed in atrial cells does this prevent or cure AF?

6. Conclusions

The major conclusion from this review is that a significant rethink of how atrial excitation contraction coupling and systolic Ca\(^{2+}\) are controlled is required in light of the demonstration that atrial cells from large mammalian species, including humans, possess a well-developed and functionally relevant T-tubule network.

6.1 Summary and perspectives for cardiac dysfunction and arrhythmias

Secondary to the initial observation above regarding the required reassessment of the fundamental mechanisms of atrial excitation contraction coupling is the gleaning of the importance of atrial T-tubules and their impact on our understanding of the mechanisms of atrial dysfunction and arrhythmias in a disease setting. Currently, our understanding of the role of atrial T-tubules in contractile dysfunction and arrhythmias is rather limited to observational studies demonstrating that in both heart failure and AF there is a loss of t-tubular structures from the atria and this occurs concurrently with the disease process. A pivotal future question is whether or not the loss of T-tubules is merely a consequence of the disease process or whether it is causative to the onset and progression of the disease. In either case, the obvious next issue is whether or not regeneration of atrial T-tubules corrects the associated disease process and restores normal cardiac function. We have also reviewed the potential role of atrial T-tubules in setting diastolic [Ca\(^{2+}\)], systolic [Ca\(^{2+}\)], response to inotropic manoeuvres such as β-AR stimulation and finally in arrhythmogenesis. With our current limited understanding of atrial T-tubule physiology and function, we have attempted to present a balanced view of the possible role for atrial T-tubules as either protective against arrhythmias or as a potential substrate for them. The final outcome of these deliberations will very much depend on our understanding of how ion channels are distributed on T-tubules and redistributed during various disease states; work that is relatively advanced in the ventricular field but at its infancy in the atria.

In summary, the recent description of T-tubules in the human atria strongly supports the use experimental models in larger mammals in order to provide genuinely translationally relevant opportunities to understand atrial physiological and pathological processes. It is clear that there is still much to do and exciting times lie ahead.

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Calcium signalling and T-tubules in the atria


