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Unique Atrial Myocyte Ca^{2+} signaling

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The transient elevation of intracellular Ca^{2+} ($[\text{Ca}^{2+}]_i$) with each heartbeat underlies the activation of contraction and contributes to diverse regulatory features of cellular and subcellular behavior. Clear differences in the details of Ca^{2+} signaling occur in different cardiac tissues and vary in the same tissue from species to species. For example, Ca^{2+} sparks, the elementary unit of Ca^{2+} signaling in heart myocytes [1,2] are plentiful during quiescent (diastolic) periods in ventricular myocytes from small rodents (e.g. rats and mice) but are rare in rabbits, dogs and humans. There are also important structural and cellular signaling differences in ventricular versus atrial myocytes. We now draw attention to the findings reported in this issue of the Journal of Molecular and Cellular Cardiology by Walden et al. (2009) who have examined the differences in Ca^{2+} signaling and in the levels of sarcoplasmic reticulum (SR) Ca^{2+} cycling proteins in atrial and ventricular myocytes [3]. They report the unexpected finding in rat heart that while the $[\text{Ca}^{2+}]_i$ transient in atrial myocytes is much smaller than in ventricular myocytes, the Ca^{2+} uptake mechanism is more robust and abundant and the SR Ca^{2+} content is greater. This may be due to the higher level of expression of sarcoplasmic reticulum (SR) Ca^{2+} -ATPase (SERCA2a) in atrial myocytes. This finding is surprising because the higher SR Ca^{2+} content might be expected to lead to Ca^{2+} overload. While such SR Ca^{2+} overload would promote arrhythmogenesis in ventricular myocytes, this is not a prominent feature of healthy atrial tissue. Here we discuss these findings and put them in context with normal and diseased atrial Ca^{2+} signaling.

Anatomic considerations of subcellular Ca^{2+} signaling in atrial versus ventricular myocytes

Atrial myocytes are thinner and have fewer transverse tubules (TTs) than do their ventricular counterparts [4,5]. They also possess smaller cell surface area and cell volume [3]. These prominent anatomic differences contribute to the unique Ca^{2+} signaling phenotype observed in healthy atrial myocytes [6]. Triggered Ca^{2+} sparks are produced at junctional SR (jSR) couplings at the surface and at jSR-TT sites. The triggering is initiated by the cellular depolarization during the cardiac action potential (AP) due to the openings of L-type Ca^{2+} channels (LTCCs). The local Ca^{2+} influx through LTCCs increases $[\text{Ca}^{2+}]_i$ in the “subspace” (the approximately 15 nm wide space between the surface sarcolemmal or TT membrane and the jSR membrane) and the elevated $[\text{Ca}^{2+}]_{\text{subspace}}$ activates the SR Ca^{2+} release

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channels (ryanodine receptors or RyR2s) in the jSR. RyR2s are organized into a paracrystalline array (or cluster of RyR2s). This local amplification of the LTCC signal is due to “Ca²⁺-induced Ca²⁺ release” or CICR and activates a Ca²⁺ spark at the jSR. During the AP, almost all Ca²⁺ sparks in ventricular myocytes are activated directly by LTCCs and thus synchronized to produce the rising phase of the [Ca²⁺]_i transient. In atrial myocytes the process is somewhat different. The [Ca²⁺]_i transient begins with the LTCC-triggered local SR Ca²⁺ release events (i.e. Ca²⁺ sparks) where the anatomy permits. The [Ca²⁺]_i transient continues as the Ca²⁺ release events are activated at other RyR2 cluster sites by CICR. The line-scan images of such slowly propagating CICR reveals a clear delay of the [Ca²⁺]_i transient deep in the atrial myocyte and the exact shape of the propagating signal depends on the extent of TT development in the atrial myocytes that are examined. The more TTs, the more rapid the deep [Ca²⁺]_i transient occurs. In ventricular myocytes no propagation delay in Ca²⁺ signal is observed [7]. Since isolated Ca²⁺ sparks in ventricular myocytes do not produce a propagating CICR signal under normal conditions, we and others had wondered what enabled such triggered Ca²⁺ sparks in atrial myocytes to produce the relatively slowly propagating Ca²⁺ signal that is normal in that tissue [8]. The findings of Walden et al. (2009) suggest that one critical factor may be the relatively elevated SR [Ca²⁺]_{SR} content in atrial versus ventricular myocytes.

Atrial-specific aspects of intracellular Ca²⁺-homeostasis

While Walden et al. (2009) indicate important differences in atrial Ca²⁺-handling proteins from those in ventricular myocytes, some of these differences also may contribute to or underlie abnormal Ca²⁺ signals in atrial myocytes in disease states (see above and below). Accumulating evidence supports the observations of Walden et al. (2009) and suggest that there are substantial differences in expression and function of Ca²⁺-handling proteins in atria compared to ventricles [9–12]. Muscle contraction and relaxation times and [Ca²⁺]_i-transient durations are shorter in atrial versus ventricular tissue of different species [12–14]. In the atria, protein levels [10,12] and activity [10,12,15] of SERCA2a are more than 2-fold higher, whereas SERCA2a-inhibitory protein phospholamban (PLN) is less expressed [10,12]. Atrial myocytes also express sarcolipin (SLN), an additional SERCA2a-inhibitory protein, that, when knocked out, enhances atrial SR Ca²⁺ uptake and contractility [16]. Atrial protein levels of RyR2 [17] and its regulators calsequestrin, triadin and junctin as well as of NCX1 [18] are lower than ventricular [10].

Atrial Ca²⁺ overload: healthy adaptive function versus prelude to subcellular Ca²⁺ alternans

As [Ca²⁺]_{SR} increases, the sensitivity of RyR2s to triggering by cytosolic [Ca²⁺]_i increases [1]. This also means that one Ca²⁺ spark is more likely to trigger another Ca²⁺ spark in atrial myocytes and remain a rare event in normal ventricular myocytes. To the extent that the SR has elevated [Ca²⁺]_{SR}, there is a more rapid propagation of the [Ca²⁺]_i transient deep in the atrial myocyte via CICR even in the absence of TTs. Under normal circumstances, this may be an adaptive mechanism that supports normal EC coupling in atrial myocytes. The thin (small diameter) character of atrial myocytes suggests that the length of propagation by CICR across the radius of the atrial myocyte is relatively small and this small distance should provide signal stability. It is, in fact, a “firewall” against runaway Ca²⁺ instability. Kirk et al. (2003) noted that in the rat, bigger atrial myocytes did tend to have more TTs and also axial components of these invaginations, identified as an ensemble called the Transverse Axial Tubule System (TATS) [5]. Thus the increase in the number of TATS elements would lessen the distance involved in the slow propagation of CICR that is generally observed in atrial myocytes. However, the benefit of the elevation of the SR Ca²⁺ content discovered by Walden et al. (2009) is that it should improve synchrony of the atrial [Ca²⁺]_i transient when TTs are absent or sparse. The disadvantage of such an elevated SR Ca²⁺ content and enhanced [Ca²⁺]_{SR} is the increased

sensitivity of the CICR process. For thin cells or large diameter cells with many invaginations in the TATS, the Ca^{2+} signal propagation is likely to be relatively stable. Large myocytes without elements of the TATS (see Fig. 1), however, will have an increased proclivity towards subcellular Ca^{2+} alternans. Since uncontrolled subcellular or cellular $[\text{Ca}^{2+}]_i$ elevations, as occurs with Ca^{2+} alternans [19–21], will activate the Na/Ca exchanger and thereby generate Na/Ca exchanger current (I_{NCX}) between APs with subsequent induction of delayed after depolarizations, the high SR Ca^{2+} content in atrial myocytes may be proarrhythmic. Such arrhythmogenic tendencies may be unmasked as cells hypertrophy and/or as elements of the TATS diminish (see Fig. 2). Additional experimental investigations and mathematical modeling should be carried out to test this hypothesis.

Evidence for a potential role of atrial Ca^{2+} overload in atrial arrhythmogenesis

The work by Walden et al. (2009) and others suggest that important differences in subcellular and global $[\text{Ca}^{2+}]_i$ signaling exist between atrial and ventricular myocytes. The greater SR Ca^{2+} content in atrial cells while “pro-arrhythmic” in principle obviously does guarantee relatively rapid $[\text{Ca}^{2+}]_i$ signaling when TTs are sparse. If, however, pathological conditions develop that increase the size of the atrial cells without TATS members (see Fig. 1A and 1B in contrast to 1C), decrease the abundance of TTs in large atrial myocytes or further increase atrial Ca^{2+} overload, it is possible that cellular and subcellular Ca^{2+} instability develops (see Fig. 2). It is also possible that an even higher SR Ca^{2+} content becomes an important contributor to atrial arrhythmogenesis [4, 22]. Abnormal Ca^{2+} signaling may thus underlie or contribute to a variety of cellular processes known to be key in atrial arrhythmogenesis (see Figure 2B). For instance, atrial SR Ca^{2+} load further increases in dogs with experimental heart failure and likely contributes to both re-entry and triggered activity that underlie heart failure-induced AF [23]. During AF-related Ca^{2+} -overload conditions in vivo, reduced inhibition of SERCA2a by phosphorylated PLN [24] helps to maintain a normal SR Ca^{2+} load in patients with AF despite the possibly increased frequencies of Ca^{2+} sparks and waves through potentially leaky (hyperphosphorylated) RyR2 channels [25, 26]. Increased spontaneous Ca^{2+} releases and Ca^{2+} waves, however, may induce delayed after depolarizations and triggered activity that may contribute to AF maintenance. In addition to complex conduction disturbances and reentry, Ca^{2+} -dependent focal sources at the cellular and subcellular levels may contribute importantly to AF pathogenesis. Full and direct experimental investigations of the relationships between local Ca^{2+} -release events, cellular Ca^{2+} dysfunction and AF, however, is still lacking. High-resolution optical mapping investigations that may discriminate between re-entry and focal activity are challenging and needed.

In conclusion, Walden et al. (2009) have carefully compared atrial to ventricular myocytes in rat heart providing important novel insights of atrial Ca^{2+} signaling. Their findings support enhanced SR Ca^{2+} content and likely increased Ca^{2+} -induced Ca^{2+} release in normal atrial myocytes. Finally these findings are consistent with the hypothesis that this increase in SR Ca^{2+} content may play an important role in the Ca^{2+} -signaling dysfunction that contributes to the pathogenesis of AF.

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Fig. 1. Transverse Axial Tubule System (TATS) in human and mouse atrial myocytes. **A** Confocal fluorescence image of a large human atrial myocyte stained with the membrane dye di-8-ANEPPS. The image reveals the exterior sarcolemmal (SL) membrane and virtually no members of the TATS. This image was taken from a patient with chronic atrial fibrillation (AF). **B.** Another (smaller) human atrial myocyte from the same sample as in panel A and imaged in a similar manner. While there are virtually no TATS members in these cells, small diameter cells such as this one should be relatively stable. It is suspected that in AF there may be a larger fraction of large TATS-deficient cells compared with control. Experimental investigation of this hypothesis is still needed. **C.** A control mouse atrial myocyte imaged as in **A** shows elements of the transverse and axial tubule system (TATS). Mouse atrial cells may

be better able to respond to the high mouse heart rate because of the atrial TATS members. (Data kindly provided by Leyla Y. Teos, James Gammie, George Rodney, Matthew Peters & W. J. Lederer, unpublished, 2008).

Fig. 2. Subcellular Ca^{2+} alternans: a possible contributing component to the development of atrial fibrillation

A. Elevated SR Ca^{2+} load in atrial myocytes promotes rapid propagation of Ca^{2+} signals in thin myocytes without elements of the TATS or in thicker atrial myocytes with an adequate number of TATS elements. In contrast the same elevation of SR Ca^{2+} in thick myocytes without TATS elements will tend to be more arrhythmogenic as it may produce subcellular Ca^{2+} alternans. B. Processes related to Ca^{2+} -homeostasis abnormalities that support the development of atrial fibrillation (AF). Subcellular Ca^{2+} alternans and SR Ca^{2+} overload (panel A) contribute directly to EADs (early after depolarizations), to DADs (delayed after depolarizations), to increased automaticity and to the effective unidirectional conduction block that may enhance

re-entry. Fibrosis and changes to conduction pathways also provide a powerful substrate that promotes re-entry development as does altered APD (action potential duration). The primary contributors to AF development are re-entry and altered automaticity. There is a clear and strong role for Ca^{2+} abnormalities in these processes.