CA SIGNALING DURING EXCITATION-CONTRACTION COUPLING IN CARDIAC MYOCYTES

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Introduction to excitation-contraction coupling of cardiac myocytes

Excitation-contraction coupling (ECC) describes the general process that links cell membrane excitation to Ca mobilization and development of contractile force in the heart. Contraction is the basic cellular function of cardiac myocytes. For the intact organ, this translates into cardiac contraction with every heart beat and pumping of blood through the systemic circulation.

Membrane depolarization activates voltage-dependent L-type calcium channels in the membrane surface, resulting in localized (sub-membrane) increases in $[Ca]_i$. Calcium that enters the cell activates SR Ca release channels (ryanodine receptors, RyRs) through a process known as calcium-induced calcium release (CICR; Fabiato, 1983). RyRs and L-type Ca channels are located in close proximity to each other in discrete signaling domains (junctional cleft, where $[Ca]_i$ can rise to tens of micromolar during activation) named Ca release units (CRU; Franzini-Armstrong and Jorgensen, 1994). The temporal and spatial summation of Ca influx and Ca release from numerous CRUs forms the global $[Ca]_i$ increase which activates the contractile machinery and initiates cell shortening. The cell then relaxes as the Ca that entered it is extruded (predominantly via Na/Ca exchange), and the Ca that was released is re-sequestered back into the SR through the action of the SR Ca ATPase (SERCA).

Although the basic features of SR Ca release are similar between atrial and ventricular myocytes, subtle differences in cellular morphology and ultrastructure are responsible for distinct differences in ECC between these two cell types. In ventricular myocyte the well developed three-dimensional network of t-tubules (Soeller and Cannell, 1999) ensures that in response to an action potential all CRUs are activated synchronously, which results in a highly uniform Ca release thoughout the entire cell volume (Cheng et al., 1994). In contrast, in atrial myocytes the t-tubular network is poorly developed or absent (Huser et al., 1996, Cordeiro et al., 2001, Mackenzie et al., 2001), and the close opposition of surface membrane Ca channels and RyRs only exists in the cell periphery (termed peripheral couplings of the junctional SR, j-SR). The SR, however, extends throughout the cell (non-junctional SR, nj-SR) and contains RyRs, capable of Ca release. During ECC, Ca release propagates from the periphery to the cell interior in a wave-like fashion via propagating CICR from CRUs of the nj-SR (McNutt and Fawcett, 1969, Kockskamper et al., 2001).

Ca sparks

Elementary Ca release events from RyRs have been visualized with a confocal microscopy and fluorescent Ca-sensitive dyes as elementary local increases in $[Ca]_i$, termed "Ca sparks" (Cheng *et al.*, 1993). Spontaneous Ca sparks correspond

to release of Ca from individual RyR clusters and occur in both ventricular and atrial myocytes. In ventricular myocytes Ca sparks have been found to colocalize with the CRUs described above, and in atrial myocytes they are observed in both peripheral (j-SR) and central (nj-SR) regions (Huser et al., 1996, Blatter et al., 1997, Woo et al., 2003, Sheehan et al., 2006).

Fig. 1 shows a sequence of high-resolution images of a representative atrial Ca spark using 2D confocal microscopy (Zeiss LSM 5 Live) and the fluorescent dye fluo-4. Ca sparks are commonly described and quantified by their frequency, amplitude (normalized increase of fluorescence above the base line, $\Delta F/F_0$), duration (measured as full duration at half maximal amplitude; FDHM), and width (measured as full width at half maximal amplitude in the longitudinal and transverse directions, FWHM). Ca sparks have a fast upstroke and a slower declining phase (see Fig. 1, *b* showing a fluorescence profile recorded from the spark center). Fig. 1, *c* shows the evolution of spatial width of a Ca spark. During the opening of the RyRs of a CRU, $[Ca]_i$ increase initially in a rather narrow domain, however as release continues Ca diffuses away from the point source of Ca, resulting in widening fluorescent signal. As release through RyRs terminates, Ca dissipates (by diffusion and reuptake) resulting in a decrease in amplitude and FWHM.

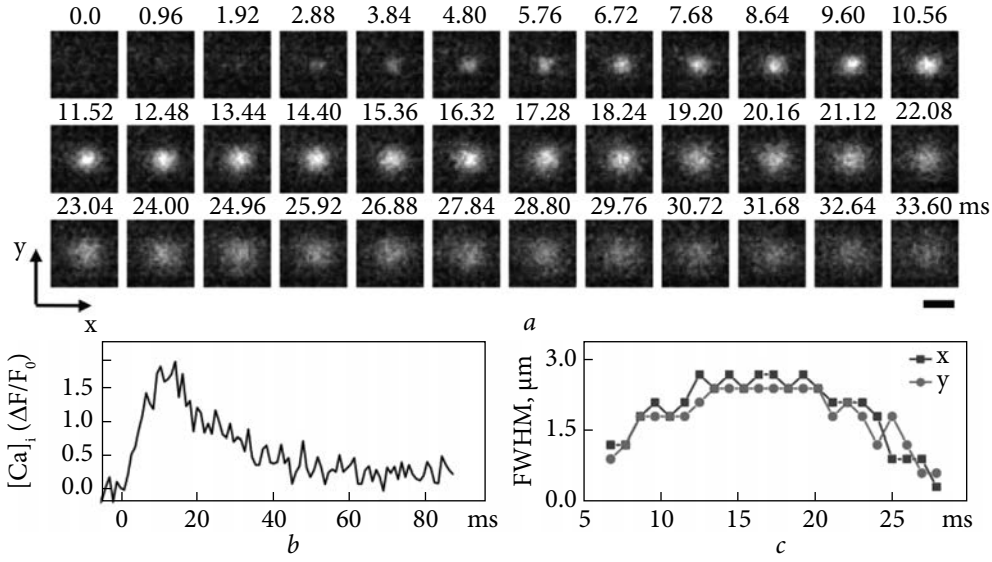

Fig. 1. Ca spark recorded from the central region of an atrial myocyte: *a* — sequential 2D confocal images of Ca spark recorded at ~1 kHz time resolution. The numbers on top of each image represents time of recording in ms (time 0.0: frame immediately preceding first detectable change in fluorescence of the Ca spark). Scale bar = 4 μm; *b* — local profile of fluorescence changes measured from a single pixel (0.3 × 0.3 μm) at the Ca spark center; *c* — time course of full width at half maximal amplitude (FWHM) along x (longitudinal cell axis) and y (transverse axis) dimension

The physiological function of spontaneous Ca sparks during diastole may represent a mechanism to stabilize SR Ca content and to prevent SR Ca overload. However, under conditions where Ca overload occurs, enhanced spark activity may be able to trigger CICR from neighboring release sites and to result in regenerative propagating and arrhythmogenic Ca waves.

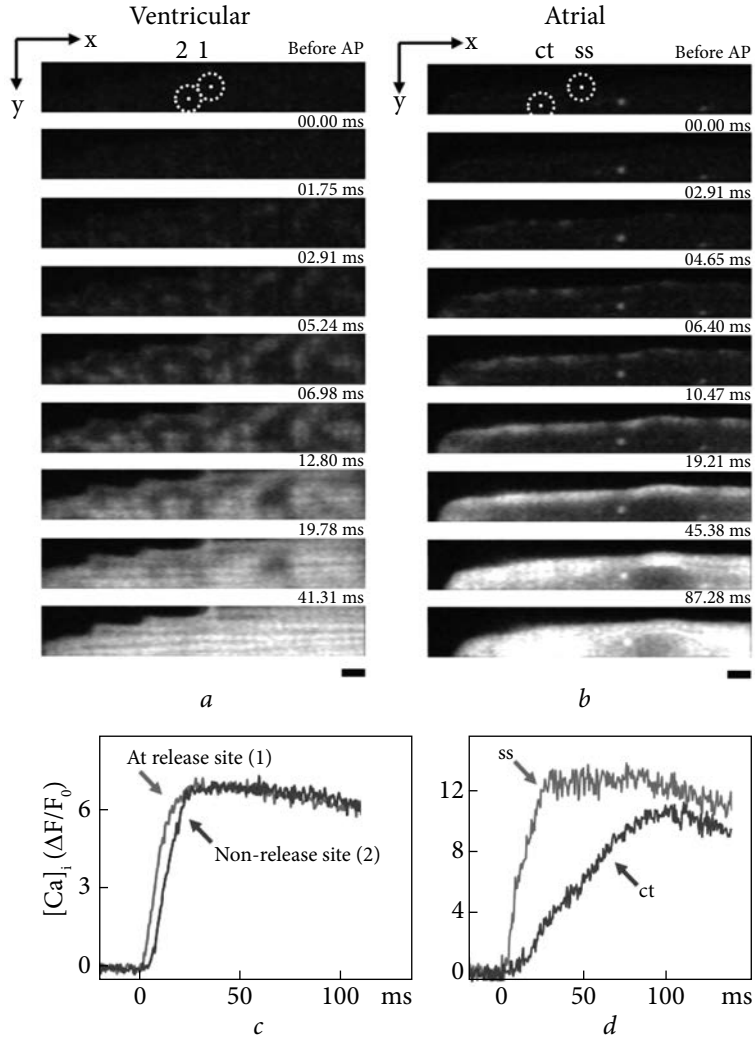

Fig. 2. $[Ca]_i$ transients recorded from the central plane of myocytes: $[Ca]_i$ transients in response to electrical field stimulation recorded from a ventricular (*a*) and atrial (*b*) myocyte visualized by 2D confocal microscopy. Local $[Ca]_i$ transients recorded from ventricular (*c*) and atrial (*d*) myocytes from a single pixel (0.3 × 0.3 μm) marked by white circles. *1*: ventricular release site; *2*: non-release site. *ss*: subsarcolemmal atrial release site in cell periphery (j-SR); *ct*: central (nj-SR) region of the cell. The numbers on top of images indicate time of recording. Scale bar is 4 μm

Action potential induced $[Ca]_i$ transient

In ventricular myocytes the action potential induced $[Ca]_i$ transient is thought to result from the spatio-temporal summation of individual Ca sparks triggered simultaneously by the action potential (Cannell et al., 1994, Cheng et al., 1996, Niggli, 1999). Fig. 2 shows a series of 2D images of depolarization induced $[Ca]_i$ transient in ventricular (A) and atrial (B) myocytes. In ventricular myocytes, initial Ca release in response to electrical field stimulation was observed as discrete

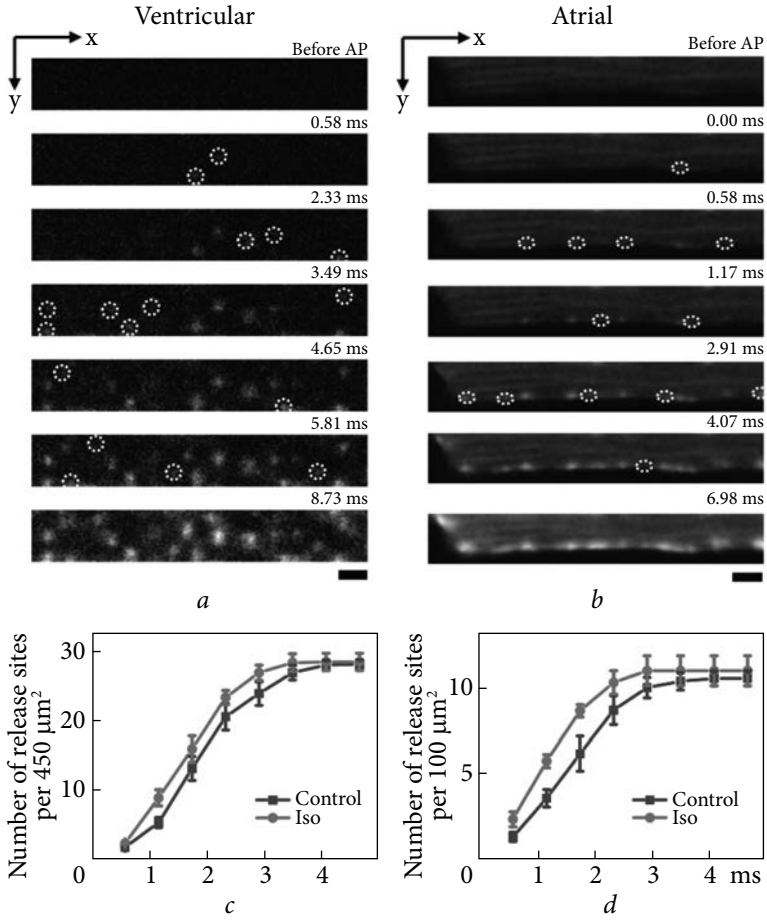

Fig. 3. Recruitment of individual Ca release sites during development of the $[Ca]_i$ transient: *a* and *b* — series of 2D confocal images of the early phase of the $[Ca]_i$ transient. White circles mark the appearance of freshly recruited release sites. The numbers on top of images indicate time of recording. Scale bar is 5 μm; *c* — cumulative number of release sites recruited during the development of the $[Ca]_i$ transient in ventricular myocytes; *d* — cumulative number of active release sites of the j-SR in atrial myocytes. Blue: control; red: after preincubation for 10 min with 1 μM of isoproterenol. AP, action potential. Time 0 ms indicates the earliest detectable release of Ca

Ca sparks originating from distinct SR release sites (CRUs) distributed throughout the myocyte resulting in a rather inhomogeneous pattern of $[Ca]_i$ at the very beginning (<10 ms) of activation. As expected, the rise of $[Ca]_i$ recorded directly from a release site precedes the rise of $[Ca]_i$ from a non-release site (where $[Ca]_i$ rises only via diffusion from sites of active release). Later during activation, the elevation of $[Ca]_i$ becomes rather homogeneous.

In atrial myocytes, the whole-cell $[Ca]_i$ transient induced by electrical stimulation, begins first with a summation of Ca release from individual sites of RyRs clusters in the peripheral j-SR membrane (Fig. 2, *b*), while Ca release from nj-SR remains silent at this point in time. While initially discrete Ca elevations can be observed, Ca release rapidly fuses into a peripheral "ring" of elevated $[Ca]_i$ (observed on average 10.1 ± 1.5 ms after the first release site becomes active). This peripheral elevation of $[Ca]_i$ forms the required gradient for Ca diffusion towards the closest Ca release sites of the nj-SR from where Ca is released via CICR. Through this reaction-diffusion mechanism, Ca release actively propagates via CICR from the periphery to the center in a Ca wave-like fashion. Due to the inherent properties of this process (dependence on Ca diffusion), the rise of $[Ca]_i$ in the cell center is delayed and often is lower in amplitude compared to the cell periphery (Fig. 2, *d*).

Fig. 3 illustrates the time course of recruitment of individual Ca release sites during ECC in ventricular (*a*) and atrial (*b*) myocytes. For this analysis, only release sites of the junctional SR were considered.

In response to electrical field stimulation, individual Ca release sites are activated asynchronously, and it takes 3-4 ms for all release sites that contribute to the global $[Ca]_i$ transient to become active. In ventricular myocytes (with j-SR throughout the cell) on average all release sites were activated after 3.9 ± 0.1 ms (Fig. 3, *b*). In atrial myocytes (considering only release from j-SR) recruitment of release sites was slightly faster (3.0 ± 0.1 ms, Fig. 3, *a*). Treatment of myocytes with the ß-adrenergic agonist isoproterenol (1 μM) accelerates recruitment of release sites, and release becomes more synchronized. The final number of active release sites, however, was not affected. ß-adrenergic stimulation modulates cardiac Ca signaling and ECC in different ways, however the enhancement of the Ca current (Jurevicius and Fischmeister, 1996) and thus the trigger for CICR is a likely mechanism to explain the improved recruitment of release sites and thus synchronization of SR Ca release. The ß-adrenergic stimulation of SR Ca uptake (stimulation of SR Ca ATPase) may facilitate this process by increasing SR Ca load.

In summary, we have revealed local (Ca sparks) and global $[Ca]_i$ transients in atrial and ventricular myocytes which were recorded with fast 2D confocal microscopy (>1000 frames per second). The comparison of atrial and ventricular myocytes shows distinct differences of ECC in the two types of cardiomyocytes. In ventricular myocytes, the opening of L- type Ca channels in response to the action potential results in Ca entry which triggers CICR from the SR simultaneously and synchronously throughout the cell due to the presence of the well developed and extensive t-tubu-

lar system. In contrast, in atrial cells lacking the t-tubular system, Ca entry triggers Ca release from the junctional SR restricted to the cell periphery, whereas subsequent delayed release from deeper regions of the cell (non-junctional SR) occurs via propagating CICR, giving raise to subcellular $[Ca]_i$ gradients during ECC.

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