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Review article

$Ca_V1.2$ sparklets in heart and vascular smooth muscle

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ABSTRACT

$Ca_V1.2$ sparklets are local elevations in intracellular Ca^{2+} ($[Ca^{2+}]_i$) resulting from the opening of a single or small cluster of voltage-gated, dihydropyridine-sensitive $Ca_V1.2$ channels. Activation of $Ca_V1.2$ sparklets is an early event in the signaling cascade that couples membrane depolarization to contraction (i.e., excitation–contraction coupling) in cardiac and arterial smooth muscle. Here, we review recent work on the molecular and biophysical mechanisms that regulate $Ca_V1.2$ sparklet activity in these cells. $Ca_V1.2$ sparklet activity is tightly regulated by a cohort of protein kinases and phosphatases that are targeted to specific regions of the sarcolemma by the anchoring protein AKAP150. We discuss a model for the local control of Ca^{2+} influx via $Ca_V1.2$ channels in which a signaling complex formed by AKAP79/150, protein kinase C, protein kinase A, and calcineurin regulates the activity of individual $Ca_V1.2$ channels and also facilitates the coordinated activation of small clusters of these channels. This results in amplification of Ca^{2+} influx, which strengthens excitation–contraction coupling in cardiac and vascular smooth muscle. This article is part of a Special Issue entitled "Calcium Signaling in Heart".

Contents

1. Introduction

On average, a heart will beat about 3 billion times during the lifetime of a human. With each beat, the heart pumps blood to the pulmonary and systemic circulation. To achieve this feat, the heart must contract in a highly coordinated fashion that allows for the sequential activation of its atria and ventricles. Ca^{2+} influx via $Ca_V1.2$ channels during the action potential (AP) serves as the signal that triggers contraction in atrial and ventricular myocytes. The chain of events that links membrane depolarization to the increase in intracellular Ca^{2+} ($[Ca^{2+}]_i$) which triggers contraction is known as excitation–contraction (EC) coupling [1]. The fidelity of EC coupling in the heart is remarkable, with every AP triggering a $[Ca^{2+}]_i$ transient and contraction [2].

The work of the heart would be futile if it were not for arteries, which deliver the blood pumped by the left ventricle to each organ in the body. Blood flow through the vasculature depends on the pressure generated by the contraction of the ventricles and the systemic resistance, which is determined by blood vessel diameter. Ultimately, the contractile state of the smooth muscle lining the walls of arteries

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controls arterial caliber and hence blood flow [3]. Like cardiac myocytes, arterial myocyte contraction is controlled by changes in membrane potential and Ca^{2+} influx via $Ca_V1.2$ channels.

Although $Ca_V1.2$ channels play a critical role in cardiac and smooth muscle EC coupling [4,5], the mechanisms by which their activation increases global $[Ca^{2+}]_i$ and initiates contraction are fundamentally different. In cardiac cells, activation of $Ca_V1.2$ channels during the AP allows a small amount of Ca^{2+} to enter the cytosol [4]. These Ca^{2+} influx events, which we are calling a "$Ca_V1.2$ sparklet" to distinguish them from sparklets produced by other Ca^{2+}-permeable channels (e.g. TRPV4, AChRs, and $Ca_V2.2$ and $Ca_V1.3$ Ca^{2+} channels), activate closely apposed ryanodine receptors (RyRs) located in the junctional sarcoplasmic reticulum (jSR) via the mechanism of Ca^{2+}-induced Ca^{2+} release (CICR) [4,6]. Accordingly, CICR amplifies the initial Ca^{2+} entry event, causing a global elevation in $[Ca^{2+}]_i$ that triggers contraction.

Unlike cardiac myocytes, an AP is not required for the activation of $Ca_V1.2$ channels in vascular smooth muscle. Rather, increases in intravascular pressure cause a graded depolarization of the sarcolemma of vascular smooth muscle that activates $Ca_V1.2$ sparklets [7,8]. In these cells, $Ca_V1.2$ sparklets are directly responsible for cell-wide increases in global $[Ca^{2+}]_i$ that activates the contractile machinery during EC coupling [8].

Optical recording of $Ca_V1.2$ sparklets has revealed interesting features about the channels underlying these events in cardiac and vascular smooth muscle cells [4,9,10]. First, the activity of $Ca_V1.2$ channels varies throughout the sarcolemma of cardiac and vascular smooth muscle. Second, the $Ca_V1.2$ channels form clusters and can gate coordinately along the sarcolemma of these cells [10–13]. In this review, we discuss the mechanisms underlying heterogeneous $Ca_V1.2$ channel activity, their cooperative activation, and the functional implications of these observations on EC coupling strength and stability under physiological and pathological conditions in cardiac and vascular smooth muscle.

2. Molecular composition and spatial organization of $Ca_V1.2$ channels in cardiac and vascular smooth muscle

$Ca_V1.2$ channels are heteromeric proteins composed of a transmembrane, pore-forming α_{1C} ($Ca_V1.2$) and accessory β, $\alpha_2\delta$-1 and, in many cells, γ subunits [14]. The expression of the auxiliary $\beta(1–3)$ subunit has been associated with enhanced channel trafficking and expression. This subunit also regulates the voltage dependencies of $Ca_V1.2$ channels [15]. $\beta2$ and $\beta3$ subunits are the predominant β subunits expressed in ventricular and vascular smooth muscle cells, respectively. Recently, the transmembrane $\alpha_2\delta(1)$ was identified as being critical for functional trafficking of $Ca_V1.2$ $\alpha1$ subunits to the plasma membrane in vascular smooth muscle [16].

The α_{1C} subunit contains four highly conserved repeat regions with variable length N terminus and a relatively long intracellular C terminus. Three α_{1C} isoforms have been identified: cardiac, $Ca_V1.2a$; smooth muscle, $Ca_V1.2b$; and neuronal, $Ca_V1.2c$. Although $Ca_V1.2a–c$ display significant sequence differences in their N and C termini, they all physically interact with the anchoring protein AKAP150/79 via their C-termini and are regulated by intracellular kinases and phosphatases to control Ca^{2+} entry and excitability [14].

Electrophysiological studies indicate that approximately 10,000–16,000 functional $Ca_V1.2$ channels are expressed in a typical ventricular myocyte. Similar approaches suggest that vascular smooth muscle cells express fewer $Ca_V1.2$ channels than ventricular myocytes (5000–10,000) [5]. However, due to differences in cell size, the average density of $Ca_V1.2$ channels cardiac and smooth muscle cells is similar (i.e., 5–15 channels/μm^2) [5,17,18].

Immunofluorescence and confocal imaging approaches have been used to determine the spatial organization of $Ca_V1.2$ channels in cardiac and vascular smooth muscle. These data suggest that $Ca_V1.2$ channels are differentially distributed throughout the sarcolemma of these cells. In cardiac myocytes, however, $Ca_V1.2$ channels are preferentially expressed in the transverse tubules (T-tubules) [19]. In vascular smooth muscle, $Ca_V1.2$ channels seem to be broadly distributed along the sarcolemma of these cells [10,20,21,22].

Raster imaging correlation spectroscopy (RICS) and *n*umber & *b*rightness (N&B) analyses have been employed to determine the rate of diffusion and cluster size of fluorescently tagged $Ca_V1.2$ channels in heterologous cells and cardiac myocytes [11]. Using RICS, the diffusion co-efficient of wild type and $Ca_V1.2$ channels carrying a mutation linked to long QT syndrome 8 (LQT8 or Timothy syndrome) was estimated to be about 2.5 $\mu m^2/s$ in these cells. N&B analysis – a moment analysis that can be used to determine the fluorescence of diffusing monomeric fluorescent molecules and to estimate the number of molecules and brightness within a volume – suggested that while most $Ca_V1.2$ (WT and LQT8) channels diffuse in solitude, clusters of 2 to 5 channels can diffuse throughout the surface membrane of tsA-201 cells expressing $Ca_V1.2$ channels fused to the tag red fluorescence protein ($Ca_V1.2$-tRFP channels) and in ventricular myocytes expressing $Ca_V1.2$-LQT8-tRFP channels (Fig. 4A). Although similar studies have not been performed in vascular smooth muscle, the observation of $Ca_V1.2$ clusters in tsA-201 and ventricular myocytes suggests this may be a general feature of these channels in excitable cells. Future studies should examine this issue in detail and also investigate the molecular mechanisms determining the size of $Ca_V1.2$ clusters in cardiac and vascular smooth muscle.

3. $Ca_V1.2$ sparklets in cardiac and vascular smooth muscle

Heping Cheng's team coined the term Ca^{2+} sparklet to name the small, local Ca^{2+} signals produced by the opening of L-type $Ca_V1.2$ channels in ventricular myocytes [4]. As mentioned above, we propose the use of the word sparklet preceded by the name of the gene encoding the Ca^{2+}-permeable channel producing the local Ca^{2+} signal to link the local Ca^{2+} to the channel that produced it. For example, we would call $Ca_V1.2$, $Ca_V1.3$, $Ca_V2.2$, AChRs and TRPV4 sparklets local Ca^{2+} signals produced by $Ca_V1.2$, $Ca_V1.3$, $Ca_V2.2$, AChRs and TRPV4 channels, respectively [10,23–26].

Since Cheng's original report, we and others, have implemented ultra fast 2-dimensional confocal and total internal reflection fluorescence (TIRF) imaging to monitor with high temporal and spatial resolution the activity of $Ca_V1.2$ sparklets in tsA201 expressing these channels, in isolated cardiac and vascular smooth muscle and in intact arteries (Fig. 1A) [9,10]. Readers interested in learning more about the strategies for the recording, detection, and analysis of sparklets will find detailed descriptions in the following publications: [12,26–30].

Implementation of these approaches revealed multiple interesting features of $Ca_V1.2$ channels in cardiac and vascular smooth muscle [4,8,10]. For example, the amplitude of $Ca_V1.2$ sparklets is quantal. In 20 mM external Ca^{2+}, the quantal unit of Ca^{2+} influx via these channels is about 40 nM (Fig. 1B). In physiological 2 mM external Ca^{2+}, the amplitude of most $Ca_V1.2$ sparklets was <20 nM or undetectable, even at very negative potentials (−70 mV), where the driving force for Ca^{2+} influx is high.

Interestingly, $Ca_V1.2$ sparklet activity is bimodal, with most of the sarcolemma being optically silent, while other regions display either low or high $Ca_V1.2$ sparklet activity [8]. $Ca_V1.2$ channel agonists increase Ca^{2+} influx by increasing $Ca_V1.2$ sparklet activity in previously silent sites and low activity sites. A $Ca_V1.2$ sparklet is always associated with an inward Ca^{2+} current (Fig. 1C). Accordingly, the signal mass of the fluorescence signal follows a linear relationship when compared to the Ca^{2+} flux through $Ca_V1.2$ channels in vascular smooth muscle. An intriguing observation made in these studies is that while the majority of $Ca_V1.2$ sparklets seem to be produced by the stochastic opening of $Ca_V1.2$ channels, a subpopulation of $Ca_V1.2$ sparklets seems to be produced by the coordinated opening of small clusters of $Ca_V1.2$ channels (Fig. 3).