frontiers in
PHARMACOLOGY

REVIEW ARTICLE
published: 06 March 2014
doi: 10.3389/fphar.2014.00035

Calcium and IP3 dynamics in cardiac myocytes: experimental and computational perspectives and approaches

Felix Hohendanner[1], Andrew D. McCulloch[2], Lothar A. Blatter[1] and Anushka P. Michailova[2]*

[1] *Department of Molecular Biophysics and Physiology, Rush University Medical Center, Chicago, IL, USA*
[2] *Department of Bioengineering, University of California San Diego, La Jolla, CA, USA*

Edited by:
Donald M. Bers, University of California, Davis, USA

Reviewed by:
Baruch Minke, The Hebrew University, Israel
Clemens Möller, Albstadt-Sigmaringen University, Germany

***Correspondence:**
Anushka P. Michailova, Department of Bioengineering, University of California San Diego, 9500 Gilman Drive, La Jolla, CA 92093-0412, USA
e-mail: amihaylova@eng.ucsd.edu

Calcium plays a crucial role in excitation-contraction coupling (ECC), but it is also a pivotal second messenger activating Ca^{2+}-dependent transcription factors in a process termed excitation-transcription coupling (ETC). Evidence accumulated over the past decade indicates a pivotal role of inositol 1,4,5-trisphosphate receptor (IP_3R)-mediated Ca^{2+} release in the regulation of cytosolic and nuclear Ca^{2+} signals. IP_3 is generated by stimulation of plasma membrane receptors that couple to phospholipase C (PLC), liberating IP_3 from phosphatidylinositol 4,5-bisphosphate (PIP_2). An intriguing aspect of IP_3 signaling is the presence of the entire PIP_2-PLC-IP_3 signaling cascade as well as the presence of IP_3Rs at the inner and outer membranes of the nuclear envelope (NE) which functions as a Ca^{2+} store. The observation that the nucleus is surrounded by its own putative Ca^{2+} store raises the possibility that nuclear IP_3-dependent Ca^{2+} release plays a critical role in ETC. This provides a potential mechanism of regulation that acts locally and autonomously from the global cytosolic Ca^{2+} signal underlying ECC. Moreover, there is evidence that: (i) the sarcoplasmic reticulum (SR) and NE are a single contiguous Ca^{2+} store; (ii) the nuclear pore complex is the major gateway for Ca^{2+} and macromolecules to pass between the cytosol and the nucleoplasm; (iii) the inner membrane of the NE hosts key Ca^{2+} handling proteins including the Na^+/Ca^{2+} exchanger (NCX)/GM1 complex, ryanodine receptors (RyRs), nicotinic acid adenine dinucleotide phosphate receptors (NAADPRs), Na^+/K^+ ATPase, and Na^+/H^+ exchanger. Thus, it appears that the nucleus represents a Ca^{2+} signaling domain equipped with its own ion channels and transporters that allow for complex local Ca^{2+} signals. Many experimental and modeling approaches have been used for the study of intracellular Ca^{2+} signaling but the key to the understanding of the dual role of Ca^{2+} mediating ECC and ECT lays in quantitative differences of local $[Ca^{2+}]$ in the nuclear and cytosolic compartment. In this review, we discuss the state of knowledge regarding the origin and the physiological implications of nuclear Ca^{2+} transients in different cardiac cell types (adult atrial and ventricular myocytes) as well as experimental and mathematical approaches to study Ca^{2+} and IP_3 signaling in the cytosol and nucleus. In particular, we focus on the concept that highly localized Ca^{2+} signals are required to translocate and activate Ca^{2+}-dependent transcription factors (e.g., nuclear factor of activated T-cells, NFAT; histone deacetylase, HDAC) through phosphorylation/dephosphorylation processes.

Keywords: Ca^{2+}, IP_3, excitation-contraction coupling, excitation-transcription coupling, cardiomyocyte

Calcium is a pivotal signaling molecule and its intracellular concentration ($[Ca^{2+}]_i$) is precisely regulated in different subcellular domains. The modulation of $[Ca^{2+}]$is a crucial factor for a variety of physiological functions of living cells. In cardiac myocytes, including ventricular and atrial cells, Ca^{2+} release through channels located in the sarcoplasmic reticulum (SR) membrane and termed ryanodine receptors (RyRs), is a key event linking membrane depolarization and mechanical activity during excitation-contraction coupling (ECC) (Bers, 2001). The amount of Ca^{2+} release with each heart beat and by that the force of contraction is also modulated by hormonal action, e.g., by Endothelin I and Angiotensin II (Proven et al., 2006). These two hormones stimulate plasma membrane receptors (G protein coupled receptors, GPCRs) that couple to phospholipase C (PLC), liberating IP_3 from phosphatidylinositol 4,5-bisphosphate (PIP_2). IP_3 freely diffuses within the cytoplasm to bind to a second type of SR Ca^{2+} release channels, the inositol 1,4,5-trisphosphate receptor (IP_3R) (Roderick and Bootman, 2003; Kockskämper et al., 2008; Berridge, 2009). IP_3Rs, albeit at a much smaller density compared to ryanodine receptors (RyR:IP_3R ~100:1), are expressed

in the SR membrane and nuclear envelope (NE) (Bootman et al., 2009). The activation of IP_3Rs upon binding of IP_3 can modulate ECC by sensitizing nearby RyRs leading to positive inotropic but also pro-arrhythmic effects (Petersen et al., 1994; Vogelsand et al., 1994; Zima and Blatter, 2004; Harzheim et al., 2009). Experimental evidence accumulated over the past decade also indicates an important role of IP_3R-mediated Ca^{2+} release in excitation-transcription coupling (ETC) and pro-hypertrophic signaling (Arantes et al., 2012). The entire PIP_2-PLC-IP_3 cascade, including GPCRs and IP_3Rs, can be found in the NE (Bkaily et al., 2011; Vaniotis et al., 2011; Tadevosyan et al., 2012). The presence of nuclear GPCRs in combination with highly localized nuclear IP_3R-mediated Ca^{2+} release and Ca^{2+} removal might provide for a putative distinct signaling domain that regulates nuclear Ca^{2+} dynamics (e.g., for autocrine signaling), whereas the cytosolic Ca^{2+} is regulated separately via sarcolemmal GPCR signaling and IP_3R-mediated SR Ca^{2+} release in conjunction with Ca^{2+} release and removal by the set of proteins involved in ECC (e.g., RyR, SERCA, troponin C). Sarcolemmal GPCRs allow for paracrine signaling and positive inotropic effects mediated by hormonal stimulation (e.g., with Angiotensin II or Endothelin I), (Kockskämper et al., 2008; Bootman et al., 2009). A comprehensive understanding of the mechanisms regulating nuclear IP_3 and Ca^{2+} signals and the impact of alterations of cytosolic Ca^{2+} and IP_3 signals on nuclear functions requires well-characterized experimental approaches, but also whole-cell system mathematical models. In this review, we discuss quantitative aspects of IP_3-dependent Ca^{2+} homeostasis in adult ventricular and atrial myocytes. In particular, we focus on novel modeling and experimental approaches to support the concept that IP_3R-mediated Ca^{2+}-release and the Ca^{2+} removal machinery in the SR and NE allow for highly localized and independent cellular signaling.

EXCITATION-CONTRACTION COUPLING IN VENTRICULAR AND ATRIAL MYOCYTES AND THE ROLE OF IP_3

In cardiomyocytes, ECC describes the process of action potential (AP) triggered Ca^{2+}-induced Ca^{2+} release (CICR) providing sufficient Ca^{2+} for the activation of the proteins regulating muscle contraction and to induce active muscle force (Bers, 2001). Membrane depolarization during an AP allows Ca^{2+} influx through voltage-dependent L-type Ca^{2+} channels (LTCC) which triggers CICR and thereby amplifies the cytosolic Ca^{2+} signal to levels required for the activation of the contractile proteins. An important feature of all *ventricular myocytes*, setting them apart from most atrial cells, is the presence of plasma membrane invaginations throughout the cytosol (transverse or t-tubules), putting LTCC in close vicinity to RyRs (**Figure 1**). The SR containing RyRs that oppose LTCC is called junctional SR (jSR). The jSR is crucial for the spatiotemporal homogeneity of Ca^{2+} release leading to largely uniform cytosolic Ca^{2+} transients ($[Ca^{2+}]_i$) during a *ventricular cell* twitch (**Figure 2**), (Franzini-Armstrong et al., 1999; Heinzel et al., 2002; Louch et al., 2004; Crossman et al., 2011; Hake et al., 2012; Signore et al., 2013). Unlike in ventricular cells, the t-tubular system in *atrial myocytes* is either absent (**Figure 1**) (Hüser et al., 1996; Kockskämper et al., 2001) or poorly developed (Kirk et al., 2003). However more recent work in sheep and human has provided evidence that atrial cells from larger animals tend to have a higher density of t-tubules (Dibb et al., 2009; Richards et al., 2011), and even in rodent atrial cells an irregular internal transverse-axial tubular system has been identified that affects kinetics of SR Ca^{2+} release (Kirk et al., 2003).The absence or paucity of t-tubules in atrial cells leads to great differences in the shape and kinetics of local Ca^{2+} transients and gradients in subcellular regions where Ca^{2+} is provided by release from jSR and non-junctional SR (njSR) (**Figure 2**). Subsarcolemmal Ca^{2+} transients rise faster, have a higher Ca^{2+} peak and are initiated by Ca^{2+} currents through LTCCs, followed by RyR-mediated Ca^{2+} release from the jSR. These local jSR Ca^{2+} transients resemble Ca^{2+} release in ventricular cells. Central cytosolic Ca^{2+} transients, however, have a slower rise time and a lower peak, and result from CICR that propagates in a Ca^{2+} wave-like fashion from the periphery to the center of the cell. (Blatter et al., 2003; Maxwell and Blatter, 2012). Furthermore, the specific topological organization of the plasma membrane in atrial myocytes leads not only to different spatial $[Ca^{2+}]_i$ distribution as compared to the ventricle, it also affects nuclear Ca^{2+} transients by further delaying their onset due to the wave-like propagation of Ca^{2+} toward the nucleus (**Figure 2**). Interestingly, for both atrial and ventricular cells, a role of cytosolic IP_3 ($[IP_3]_i$) has been reported for the modulation of cytosolic Ca^{2+} transients in a variety of animal models (Zima and Blatter, 2004; Proven et al., 2006; Domeier et al., 2008; Harzheim et al., 2009; Kim et al., 2010). IP_3R channel activity, with type-2 IP_3Rs as the most prevalent isoform in cardiac myocytes, depends on $[IP_3]_i$ and $[Ca^{2+}]_i$ (Michell et al., 1981; Domeier et al., 2008; Kockskämper et al., 2008). There is evidence that atrial myocytes express functional IP_3Rs at higher densities than ventricular myocytes (**Figure 1**; in ventricular cell the IP_3Rs are not shown in the junctional space due their relatively low density) (Mackenzie et al., 2004; Zima and Blatter, 2004). As shown in **Figure 2**, the acute increase in cytosolic IP_3, induced by photolytic release of IP_3 from a caged IP_3 compound, increases cytosolic Ca^{2+} transient peak amplitudes during field stimulation in atrial cells in contrast to ventricular cells. In ventricular cells only increased expression levels of IP_3R, as it occurs in cardiac hypertrophy, could experimentally be tied to enhanced cytosolic SR Ca^{2+} release (Harzheim et al., 2009). The neurohumoral stimulation with Endothelin I or Angiotensin II, however, has been shown to have similar positive inotropic effects in both ventricular and atrial cells, indicating a role of IP_3-mediated Ca^{2+} release in the enhancement of cytosolic Ca^{2+} release (Zima and Blatter, 2004).

EXCITATION-TRANSCRIPTION COUPLING IN VENTRICULAR AND ATRIAL MYOCYTES AND THE ROLE OF IP_3

Nuclear Ca^{2+} signals however are different with regards to kinetics during action potential induced Ca^{2+} transients. This can largely be attributed to the fact that the nucleus is surrounded by the nuclear envelope (Kockskämper et al., 2008; Alonso and García-Sancho, 2011), consisting of the outer and inner nuclear membranes and the space between them that is contiguous with the SR (Wu et al., 2006; Shkryl et al., 2012). The nuclear membranes fuse at many locations to form pores (diameter ~100, length ~50 nm) that harbor the nuclear pore complexes (NPCs).

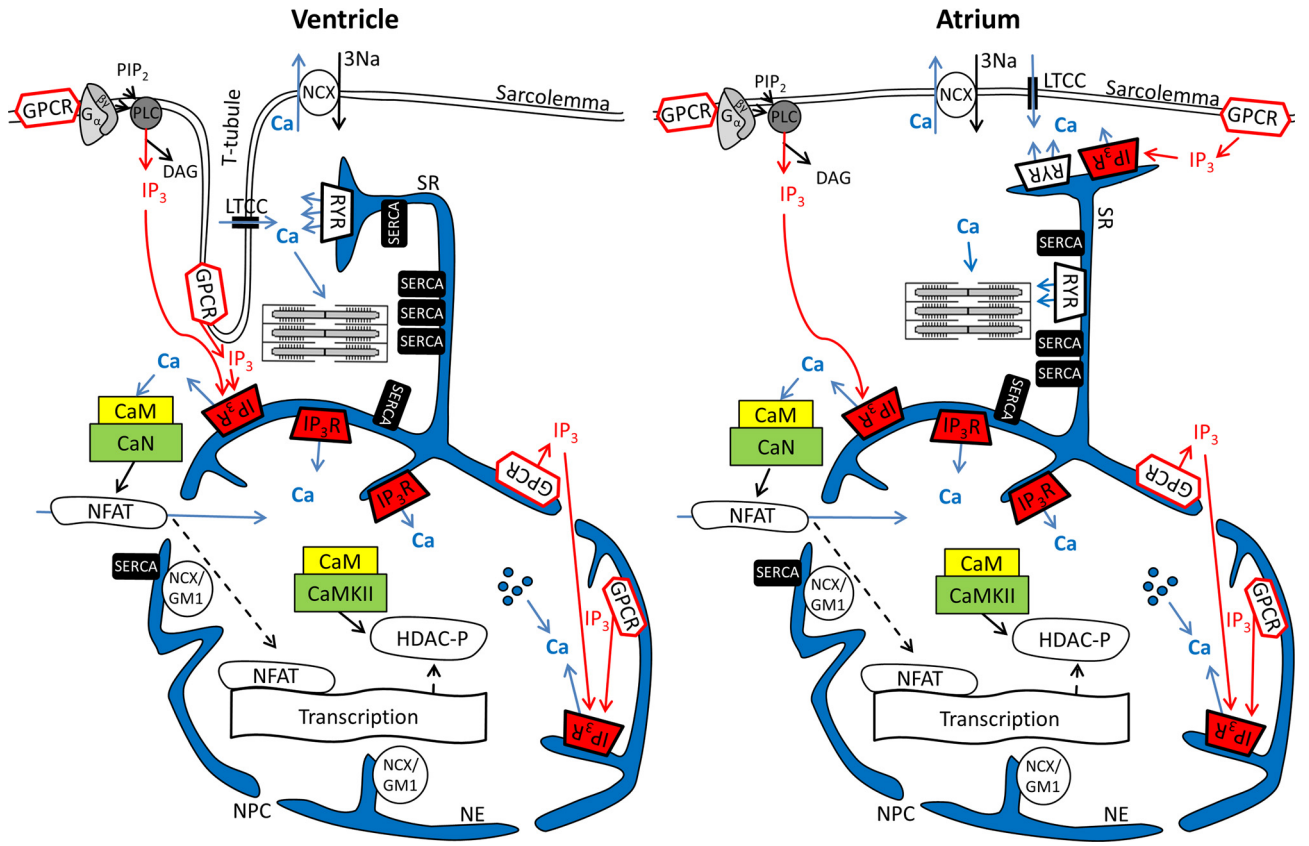

FIGURE 1 | Ca^{2+} and IP_3 and their involvement in cardiac excitation-contraction and excitation-transcription coupling in ventricular and atrial myocytes. Schematics depict parts of the sarcolemmal plasma membrane as well sarcoplasmic reticulum and nuclear envelope as a contiguous Ca^{2+} store. Abbreviations: GPCR, G protein-coupled receptor; PIP_2, phosphatidylinositol 4,5-bisphosphate; PLC, phospholipase C; LTCC, L-type Ca^{2+} channel; NCX, Na^+/Ca^{2+} exchanger; RyR, ryanodine receptor; IP_3R, IP_3 receptor; SERCA, SR Ca^{2+} ATPase; NCX/GM1, NCX ganglioside complex; CaM, Calmodulin; CaN, Calcineurin; CaMKII, Ca-Calmodulin dependent kinase; NFAT, nuclear factor of activated t-cells; DAG, Diacylglycerol; HDAC, histone deacetylase; NPC, nuclear pore complex; Ca^{2+} released from intra-nuclear pools.

The NPCs are the major gateway for ions (including Ca^{2+}) to diffuse along the gradient between the cytosol and nucleoplasm. It has been proposed that NPCs can act as diffusion filter and introduce a kinetic delay in the equilibration of nucleoplasmic Ca^{2+} concentration ($[Ca^{2+}]_{nuc}$) and $[Ca^{2+}]_i$ (Bootman et al., 2009). The extent of the kinetic delay might be subject to modulation. Although NPCs do not close, their conductance can change in response to factors such as Ca^{2+} and ATP. The density of NPCs can vary from 1 to 5 NPCs per μm^2, depending on the cell type (Wang and Clapham, 1999). A greater expression of NPCs would allow for a more rapid equilibration of $[Ca^{2+}]_i$ and $[Ca^{2+}]_{nuc}$. Recent data from Alonso and García-Sancho (2011) also suggest a role for NE invaginations (nucleoplasmic reticulum) and intra-nuclear Ca^{2+} pools for the regulation of nuclear Ca^{2+} (**Figure 1**). More evidence that nuclear Ca^{2+} dynamics are not just a function of cytosolic Ca^{2+} transients can be found in structural and functional differences of NE Ca^{2+} handling proteins as compared to the SR. Even though the NE is an extension of the SR (Wu et al., 2006; Shkryl et al., 2012) SERCA presumably is not expressed at the inner NE membrane (Malviya and Klein, 2006; Bootman et al., 2009). Nonetheless, other putative Ca^{2+} handling and ion transporting proteins have been suggested to be present in the NE, including a splice variant of the type-1 Na^+/Ca^{2+} exchanger associated with ganglioside (NCX/GM1 complex, typical for non-excitable cells), RyRs, NAADPR (nicotinic acid adenine dinucleotide phosphate receptor), Na^+/K^+ ATPase and Na^+/H^+ exchanger (Gerasimenko et al., 2003; Irvine, 2003; Bkaily et al., 2006; Ledeen and Wu, 2007; Zima et al., 2007; Guatimosim et al., 2008; Wu et al., 2009).

Even more important seems the preferential expression of IP_3Rs in the NE (Bare et al., 2005). Using Fluo-5N Zima et al. observed a depletion of the nuclear envelope upon experimental stimulation of IP_3Rs with IP_3 in isolated nuclei (Zima et al., 2007) that was paralleled by an increase of $[Ca^{2+}]_{nuc}$. Wu and colleagues obtained similar results with Fluo-5N on IP_3 dependent NE Ca^{2+} depletion in permeabilized cells (Wu et al., 2006). The importance of IP_3 for the regulation of $[Ca^{2+}]_{nuc}$ is underscored by the results shown in **Figure 2**: Following cell-wide IP_3 uncaging, nuclear Ca^{2+} transients are consistently and preferentially altered in atrial and ventricular cells. However since IP_3 is buffered (i.e., by IP_3Rs) and degraded over time (Woodcock and Matkovich, 2005), the subcellular localization of IP_3Rs and the site of IP_3

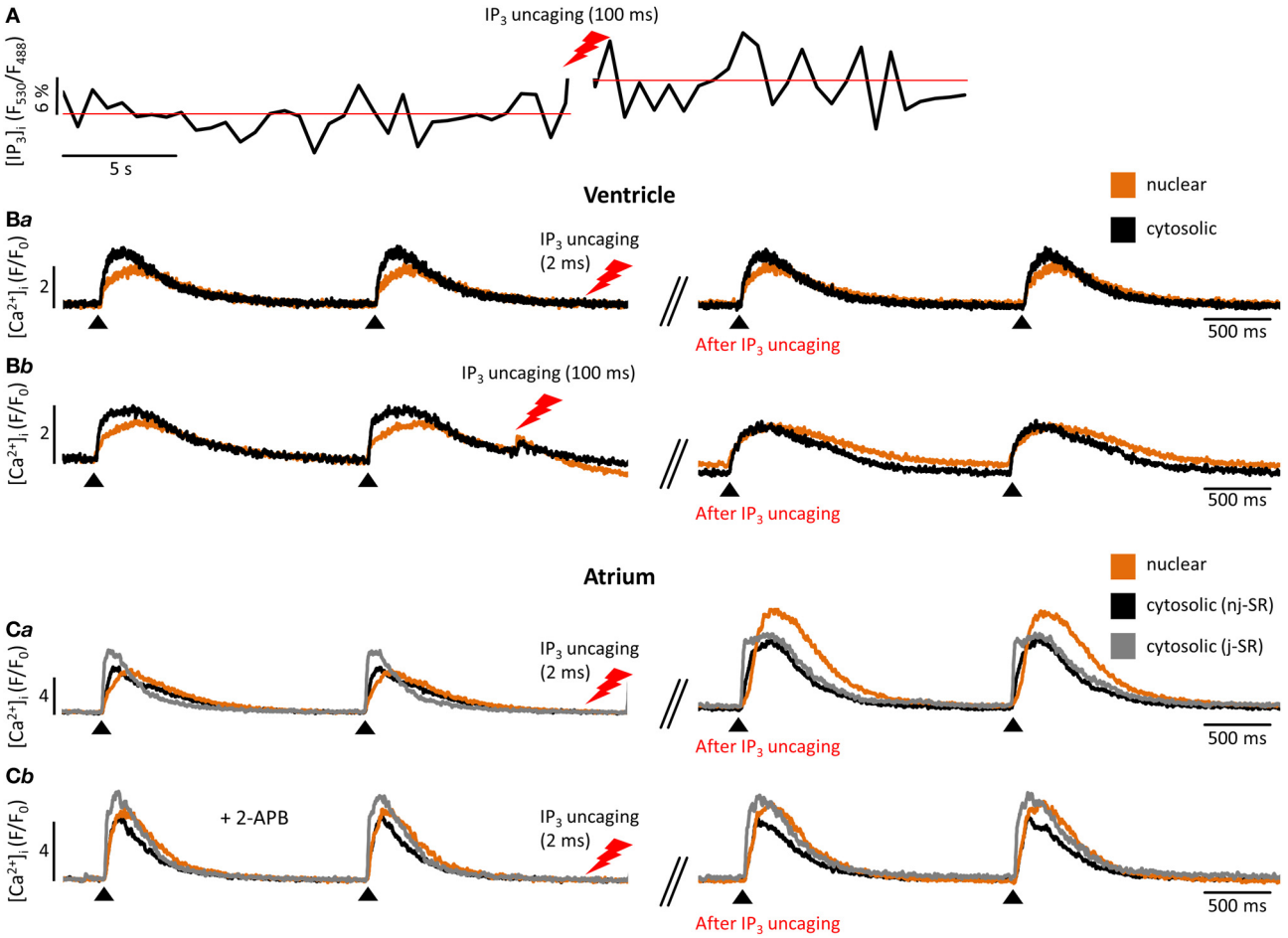

FIGURE 2 | Experimental measurement of cytosolic and nuclear Ca^{2+} and IP_3. **(A)** Cardiomyocyte loaded with the caged IP_3 compound cag-IP_3 PM and expressing cytosolic FIRE-1. Whole cell [IP_3] signal shown as changes in FIRE-1 FRET signal (expressed as F_{530}/F_{488}) after global IP_3 uncaging by exposure to 405 nm laser illumination for 100 ms. **(B*a*,*b*)** Effects of global IP_3 uncaging (2 ms or 100 ms illumination) in field-stimulated ventricular myocytes on global nuclear and cytosolic Ca^{2+} transients. **(C*a*)** The effect of global IP_3 uncaging (2 ms) on global nuclear, subsarcolemmal (j-SR) and central (nj-SR) Ca^{2+} transients in field stimulated atrial myocyte. **(C*b*)** The effect of global IP_3 uncaging (2 ms) on global nuclear, j-SR and central Ca^{2+} transients in field stimulated atrial myocyte pre-incubated with the IP_3R blocker 2-APB. Pacing frequency 0.5 Hz. Black arrowheads indicate application of electrical stimuli.

generation (i.e., GPCR) are important to generate highly localized Ca^{2+} signals to control Ca^{2+}-dependent transcription (Bers, 2013; Ibarra et al., 2013). The traditional view on the positioning of GPCRs in cardiac myocytes sees their main site of expression in the sarcolemmal and nuclear membrane (**Figure 1**). Only recently, work from Ibarra et al. (2013) suggested a third type of localization for GPCRs in t-tubules close to the nuclear envelope (**Figure 1**, ventricular cell). The positioning of IP_3 production and IP_3Rs is important since differences in the kinetics of local [Ca^{2+}] can lead to altered activation of transcription factors. A pronounced local elevation of [Ca^{2+}] for instance, can activate calmodulin dependent-protein kinase II (CaMKII) and promote histone deacetylases (HDAC) phosphorylation (Wu et al., 2006), whereas a sustained smaller [Ca^{2+}] elevation increases nuclear factor of activated T-cells (NFAT) dephosphorylation via the Ca^{2+} sensitive phosphatase calcineurin (CaN). This ultimately leads to the activation of different sets of transcription factors, e.g., myocyte enhancer factor 2 (MEF2) for HDAC and GATA for NFAT (Molkentin et al., 1998). The separate set of Ca^{2+} release and removal proteins in the NE, with IP_3Rs as the most prominent example, as well as the specific expression of GPCRs in the sarcolemmal and nuclear membranes might be key to understanding the conundrum of Ca^{2+} being a modulator of contraction and transcription at the same time (Bootman et al., 2009). Mathematical modeling of nuclear and cytosolic Ca^{2+} homeostasis, accounting for different expression levels of sarcolemmal, cytosolic and nuclear Ca^{2+} handling proteins, paralleled by experimental approaches might provide a better understanding of functional differences of nuclear and cytosolic Ca^{2+}.

EXPERIMENTAL TOOLS FOR MEASURING CYTOSOLIC AND NUCLEAR Ca^{2+} AND IP_3 SIGNALS

Confocal laser microscopy, multiphoton imaging and conventional microscopy provide the basis for visualization of whole cell and subcellular ion concentration distributions, and the development of chemical fluorescent Ca^{2+} indicators

(Grynkiewicz et al., 1985) made imaging of Ca^{2+} movements inside living cells feasible. Nowadays a variety of ratiometric and non-ratiometric Ca^{2+} indicators, with Indo-1 and Fluo-4 among the most prominent examples, are being used. In principle, upon excitation, these indicators emit light at particular wave lengths and the emitted fluorescence intensity or the emission spectrum is changed in a Ca^{2+} bound state (Takahashi et al., 1999). The dissociation constant (K_d) as a measure of Ca^{2+} binding affinity is crucial for the selection of the appropriate Ca^{2+} dye for a particular cellular compartment of interest. Low affinity, high K_d dyes (like Fluo-5N) are used for the visualization of changes in SR $[Ca^{2+}]$ or nuclear envelope $[Ca^{2+}]$, whereas, e.g., Fluo-4 (K_d of 345 nM) is one of the preferred dyes for imaging of changes in cytosolic free $[Ca^{2+}]$, which varies roughly between 100 nM and values at times exceeding 1 μM during ECC. Since the nucleoplasm and the cytoplasm are interconnected compartments with similar global $[Ca^{2+}]$ characteristics, dyes suitable to show changes in $[Ca^{2+}]_i$ can be used for the detection of changes in $[Ca^{2+}]_{nuc}$ as well. Using Ca^{2+} sensitive dyes, Zima and Blatter (2004) were able to visualize cytosolic IP_3R-mediated Ca^{2+} release events (Ca^{2+} puffs) and show a positive inotropic effect of neurohumoral stimulation with Endothelin-1 in cardiac myocytes. As mentioned above, the same group was also able to show changes of local nuclear envelope $[Ca^{2+}]$ in isolated nuclei upon stimulation with IP_3, using Fluo-5N (Zima et al., 2007).

A variety of pharmacologic interventions can be used to influence the IP_3-dependent signaling cascade. Tools for stimulation of the neurohumoral GPCR pathway in cardiomyocytes include for example Angiotensin II and Endothelin-1. PLC-inhibitors like U73122 and IP_3R blockers like 2-Aminoethoxydiphenyl borate (2-APB) or heparin are widely used IP_3R blockers to study the GPCR/PLC/IP_3 pathway. More recent molecular techniques and the generation of transgenic animals complement these tools. Noteworthy are the generation of IP_3R knock-out and IP_3R overexpressing mice as well as the development of IP_3-sponges that allows the cellular overexpression of IP_3 buffering proteins. The generation of IP_3R overexpressing mice combined with the adenoviral expression of an IP_3 sponge provided novel insights into the importance of this pathway in cardiac physiology and pathophysiology. Ca^{2+} transients in IP_3R overexpressing mice were increased and showed a higher potential for arrhythmias after Endothelin-1 treatment. These effects were abrogated after expression of the IP_3 sponge (Nakayama et al., 2010). Insensitivity toward GPCR stimulation and IP_3R-mediated pro-arrhythmic effects were confirmed in IP_3R knock-out mice (Li et al., 2005).

An approach to directly visualize cellular $[IP_3]$ would allow for a more complete picture of cell physiology. Only recently Remus et al. (2006) developed biosensors termed FIRE to dynamically study $[IP_3]$ in living cells. Briefly, FIRE is incorporated into an adenoviral vector, expressed in target cells, and utilizes fluorescence resonance energy transfer (FRET) between cyan and yellow fluorescent protein (CFP and YFP) upon binding of IP_3. For that purpose FIRE contains a fusion protein of CFP, YFP, and the IP_3 binding domain of the IP_3 receptor type 1, 2, or 3 and can be targeted to the cytosolic or nuclear compartment. An increase in $[IP_3]$ is detected by an increase in FRET signals and a change in the YFP/CFP fluorescence ratio.

Further progress in the study of IP_3-dependent Ca^{2+} signaling became possible with the development of caged IP_3 compounds (Smith et al., 2009). Upon UV-light dependent photolysis, IP_3 is released in its biological active form and can be readily used to study this signaling pathway without possible additional effects of GPCR stimulation other than IP_3 generation (i.e., effects mediated by diacylglycerol that is generated concomitantly with IP_3 by PLC). These approaches can be used in parallel, as shown in **Figure 2A**: a cardiomyocyte expressing FIRE-1-cyt exhibits an increase in the FRET signal of ∼6% upon IP_3 uncaging, indicative of a detectable change of global cytosolic $[IP_3]$. Moreover **Figure 2** depicts the influence of IP_3 uncaging on different cellular compartments in atrial and ventricular cells. **Figure 2B** exemplifies the small impact of IP_3 uncaging on local cytosolic and nuclear Ca^{2+} transients in field stimulated (0.5 Hz) ventricular cells (Fluo-4). Only prolonged exposure to the IP_3 uncaging signal (100 ms laser illumination) has immediate visible effects on local Ca^{2+} release (**Figure 2C**). The IP_3 effects on diastolic $[Ca^{2+}]_i$ and the Ca^{2+} transient amplitude are particularly pronounced for the nuclear region. As compared to ventricular cells, atrial myocytes are more sensitive to IP_3 uncaging at smaller laser exposure durations (2 ms; i.e., smaller $[IP_3]$) and the overall effect on cytosolic, nuclear and subsarcolemmal Ca^{2+} transient amplitudes is higher upon IP_3 uncaging. Note also the altered Ca^{2+} transient kinetics with a prolongation of the Ca^{2+} transient's amplitude following IP_3 uncaging (**Figure 2C*a***). **Figure 2C*b*** shows the effect of the IP_3R blocker 2-APB (10 μM). The effect of IP_3 uncaging on Ca^{2+} transients in an atrial cell, pre-incubated with 2-APB, was abolished.

MATHEMATICAL APPROACHES FOR SIMULATING CYTOSOLIC AND NUCLEAR Ca^{2+} AND IP_3 SIGNALS

Computational modeling has proven to be a powerful approach to study cardiac physiology and its implications for disease. With increasing availability of biophysical and physiological data, mathematical models have also become more sophisticated. They provided new insights into how cellular structures, channels and receptor distributions or Ca^{2+}/IP_3 signaling regulate cardiac ECC. A number of *deterministic models* of ventricular and atrial myocyte electrophysiology, intracellular Ca^{2+} handling and bioenergetics have been published. For a more complete review on successes and failures in these modeling pursuits we refer the reader to some excellent recently published articles (Noble, 2011; Jafri, 2012; Noble et al., 2012; Sobie and Lederer, 2012; Poláková and Sobie, 2013; Wilhelms et al., 2013). Several *computational models* have been constructed to investigate IP_3 synthesis and the sub-cellular mechanisms regulating IP_3R-mediated Ca^{2+} signaling. The first model of an IP_3 signaling system, built to simulate IP_3 signals in response to stimulation with cardiac hypertrophic neurohumoral agonists like Endothelin-1 and Angiotensin II, was published by Cooling et al. (2007). The key controlling parameters with respect to the resultant cytosolic $[IP_3]$ in atrial cells were identified, including phosphorylation of membrane receptors, ligand strength, binding kinetics to pre-coupled (with GαGDP) receptors and kinetics associated with pre-coupling the receptors. In 1992, De Young and Keizer (1992) constructed the first simplified model of the IP_3 receptor. Subsequent theoretical studies,

based on new experimental data, have investigated the complex dynamic properties of type 1, 2, or 3 IP_3Rs (Li and Rinzel, 1994; Laurent and Claret, 1997; LeBeau et al., 1999; Moraru et al., 1999; Mak et al., 2001; Sneyd and Dufour, 2002; Dawson et al., 2003; Siekmann et al., 2012). Based on quantitative measurements of IP_3R properties, several stochastic models of the single channel and channel-clusters have been constructed (Swillens et al., 1998; Shuai and Jung, 2002; Falcke, 2003; Fraiman and Dawson, 2004; Thul and Falcke, 2004; Gin et al., 2009). Fraiman and Dawson (2004) were the first to include an explicit dependence of IP_3R gating on SR-luminal Ca^{2+}. To investigate the mechanisms underlying pacemaker cell activity, Youm et al. (2006) developed a deterministic model that includes ion channels, NCX, pumps, the intracellular machinery for Ca^{2+} regulation, cytosolic IP_3 production and IP_3-mediated Ca^{2+} release activity. Their model supports the idea that the cyclic changes in cytosolic Ca^{2+} and IP_3 play a key role in the generation of regenerative pacemaker potentials. *Spatiotemporal continuum models,* seeking to investigate the mechanisms of IP_3-mediated Ca^{2+} signaling in cells where IP_3Rs are known to be the dominant Ca^{2+} release channels, have been published as well. Jafri and Keizer, combining a realistic model of IP_3-induced Ca^{2+} oscillations with the diffusion of IP_3 and buffered diffusion of Ca^{2+}, developed a reaction-diffusion continuum model in Xenopus oocytes (Jafri and Keizer, 1994, 1995). Their results suggest that Ca^{2+} diffusion, which was much slower than that of IP_3 because of endogenous Ca^{2+} buffers, had only a small effect on predicted Ca^{2+} transients. These findings imply a possible previous undisclosed role for IP_3 in cell signaling. Means et al. (2006) used a reaction-diffusion model to simulate Ca^{2+} and IP_3 dynamics in mast cells. The model was built upon a 3D reconstruction of the endoplasmic reticulum (ER) geometry from electron-tomography series. This model simultaneously tracks the changes in cytoplasmic and ER $[Ca^{2+}]$, includes luminal and cytoplasmic Ca^{2+} buffers, plasma membrane Ca^{2+} fluxes, SERCA, ER leakage, and type-2 IP_3R. A unique feature of the model is the inclusion of the stochastic behavior of type-2 IP_3R. The results showed that IP_3Rs in close proximity modulate the activity of their neighbors through local Ca^{2+} feedback effects. Finally, in 1999 an analysis performed by fluorescence measurements of $[Ca^{2+}]_i$ and $[Ca^{2+}]_{nuc}$ in ventricular myocytes revealed that $[Ca^{2+}]_{nuc}$ increases concomitantly with $[Ca^{2+}]_i$ upon electrical stimulation, but the pattern of $[Ca^{2+}]_{nuc}$ increase was biphasic (rapid and slow) (Genka et al., 1999). Both sets of $[Ca^{2+}]_i$ and $[Ca^{2+}]_{nuc}$ data were well fitted by predictions derived from a simplified model of Ca^{2+} diffusion across the NPCs with two different Ca^{2+} diffusion constants. A plausible explanation of this finding is that the change in $[Ca^{2+}]_{nuc}$ is caused by Ca^{2+} diffusion from the cytosol to the nucleus through NPCs, but the permeability of the NPCs shifts from free to moderately restricted during contraction (Genka et al., 1999). The partial restriction of Ca^{2+} diffusion into the nucleus at high $[Ca^{2+}]_i$ may support the idea of a defense mechanism protecting the nucleus against Ca^{2+} overload during cell contraction.

Taken together, the aforementioned modeling efforts fill a number of specific gaps of knowledge with respect to cell electrophysiology and cytosolic Ca^{2+} and IP_3 signaling. To date, however, no quantitative model coupling the cell electrophysiology with Ca^{2+} and IP_3 signaling in the cytosol and nucleus in cardiomyocytes exists. The development of a new system model, coupling ECC and ETC is important because: (a) this tool would provide fundamental new information on the role of IP_3R-mediated Ca^{2+} signaling during ECC for arrhythmogenesis, for electrophysiological changes and for nuclear Ca^{2+} signaling in normal and failing cardiac cells; (b) as more experimental details on the complexity of IP_3 regulation in myocytes accumulates, the intuitive interpretation of new findings becomes increasingly impractical and sometimes controversial. In pursuing this goal we extended the Shannon-Bers model in rabbit ventricular myocytes (Shannon et al., 2004). New equations, describing nuclear Ca^{2+} dynamics and its dependence on $[Ca]_i$, nuclear Ca^{2+} buffering and transport via NPCs and NE (i.e., SR) were incorporated (see **Figure 1**; Michailova et al. unpublished data). Preliminary results (**Figures 3A,B**) show that the model predictions are in qualitative agreement with our Ca^{2+} transient measurements at 0.5 Hz electrical stimulation (see **Figure 2B**) and published experimental data (Ljubojevic et al., 2011) of global cytosolic and nuclear Ca^{2+} transients under control conditions, i.e., in absence of activation of IP_3 signaling. The predicted $[Ca^{2+}]_i$ and $[Ca^{2+}]_{nuc}$ transients (and action potentials and $[Ca^{2+}]_{SR}$; not shown) are stable during 10 min stimulation at 0.5, 1, or 2 Hz. The model mimics also the frequency-dependent increases in the diastolic $[Ca^{2+}]_i$ (Shannon et al., 2004), but no obvious differences in diastolic levels of $[Ca^{2+}]_{nuc}$ vs. $[Ca^{2+}]_i$ at any given frequency were predicted. At each frequency the systolic Ca^{2+} peaks were lower in the nuclei and positive force-frequency increases in systolic $[Ca^{2+}]_i$ and $[Ca^{2+}]_{nuc}$ were predicted. The kinetic parameters of Ca^{2+} transients (time to peak and time to 50% $[Ca^{2+}]$ relaxation; RT_{50}) were slower in the nucleus as compared to the cytosol. The physiological utility of the model was tested further by applying different frequencies to simulate the positive force-frequency relationship (**Figure 3C**). In agreement with experiments (Ljubojevic et al., 2011), upon increasing the rate from 0.5 to 2 Hz diastolic $[Ca^{2+}]$ and systolic Ca^{2+} peaks in the nucleus and cytoplasm increased in magnitude and the predicted amplitude of the Ca^{2+} transients were smaller in the nucleus compared to the cytosol.

CONCLUSIONS AND FUTURE PERSPECTIVES

In this review we discussed the current state of experimental and modeling approaches to investigate nuclear and cytosolic Ca^{2+} homeostasis, whereby we focused on IP_3-dependent Ca^{2+} signaling in adult myocytes. We presented experimental data from ventricular and atrial cells, showing the effects of sudden increases in $[IP_3]$ on nuclear and cytosolic Ca^{2+} transients during field stimulation as well as different approaches to study IP_3-mediated Ca^{2+} release (i.e., FIRE-1-cyt as a tool to quantify $[IP_3]$, IP_3 uncaging to mimic physiological increases in $[IP_3]$ and 2-APB to block IP_3R mediated Ca^{2+} release). Moreover we compared experimentally the influence of IP_3 uncaging on different compartments (nucleoplasm, cytosol) and were able to show that ventricular cells need a stronger IP_3 stimulus to elicit a nuclear response, whereas atrial cells display substantial increases in nuclear and cytosolic Ca^{2+} transient amplitude upon a weaker IP_3 uncaging stimulus, consistent with their higher total expression of IP_3Rs as compared to ventricle. The recent development of FRET-based

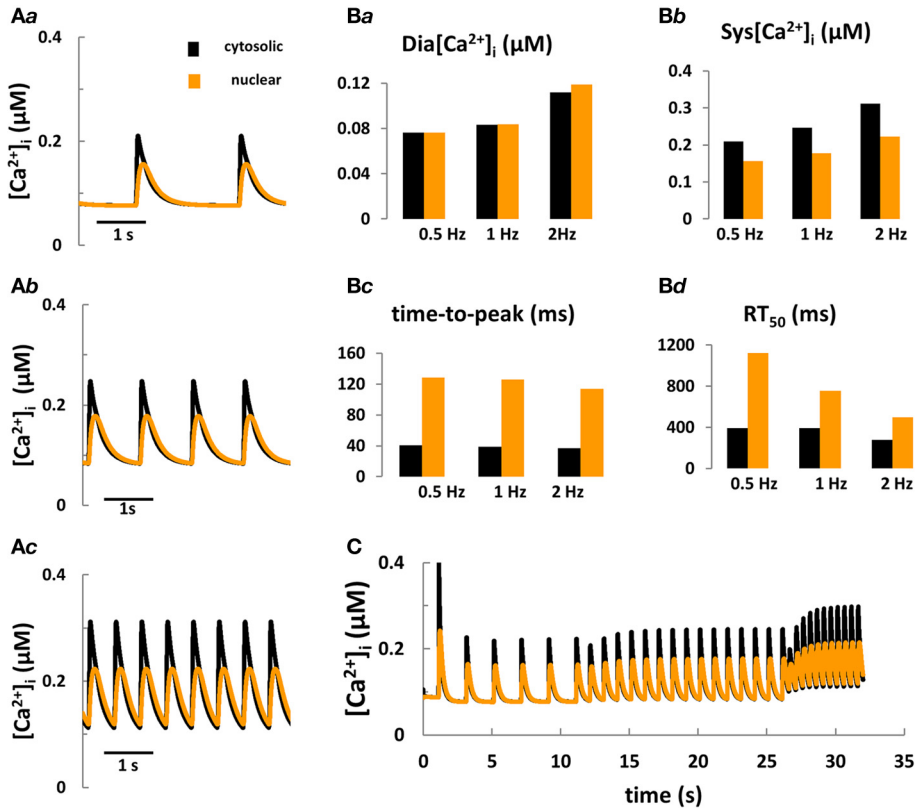

FIGURE 3 | Computer modeling of cytosolic and nuclear Ca^{2+} signals under control conditions. (**A*a*–A*c***) Predicted cytosolic and nuclear steady-state Ca^{2+} transients under control conditions in rabbit ventricular myocytes stimulated for 10 min at frequencies of 0.5, 1, and 2 Hz, respectively. **(B*a*–B*d*)** Predicted kinetic parameters of $[Ca^{2+}]_i$ and $[Ca^{2+}]_{nuc}$ transients: Dia $[Ca^{2+}]$, diastolic Ca^{2+}; Sys $[Ca^{2+}]$, systolic Ca^{2+}; RT_{50}, time to 50% $[Ca^{2+}]$ relaxation. **(C)** Frequency-dependent changes in $[Ca^{2+}]$ in the nucleus vs. the cytoplasm during stepwise increases of the stimulation frequency from 0.5 to 2 Hz.

probes used for the detection of $[IP_3]$ as well as approaches to alter nuclear and/or cytosolic $[IP_3]$ provide experimental tools for the study of IP_3-dependent Ca^{2+} release and its importance in ECC and ETC.

We also presented our recent efforts of a first attempt to develop an electrophysiological and Ca^{2+} signaling model that integrates three different cellular subsystems (cytosol, SR, nucleus) and couples Ca^{2+} dynamics in the cytosol and nucleus. This new tool is under development and will undergo further testing in its prediction of experimental $[Ca^{2+}]_{nuc}$ and $[Ca^{2+}]_i$ data in rabbit ventricular cells. The proposed model will also be extended to investigate how the complex dynamics of type-2 IP_3 receptors (Sneyd and Dufour, 2002; Siekmann et al., 2012), the stochastic behavior of IP_3R channel (Fraiman and Dawson, 2004) and/or the stimulation of IP_3 signal transduction pathway with neurohumoral agonists (Cooling et al., 2007) regulate ventricular ECC and ETC. Furthermore, the mechanisms underlying IP_3-induced positive inotropy in cardiomyocytes continue to be controversial with numerous cellular targets being implicated in the response, including L-type Ca^{2+} channels, K^+ channels, and Na^+/Ca^{2+} exchange (Lauer et al., 1992; Watanabe and Endoh, 1999; Woo and Lee, 1999; Yang et al., 1999; He et al., 2000; James et al., 2001; Zhang et al., 2001; Puglisi et al., 2011; Signore et al., 2013). The current model can be extended to investigate these effects as well. This model also provides a good quantitative framework to integrate reactions for calmodulin (CaM), calcineurin (CaN), CaMKII, and CaM buffering in the nucleus and can be coupled to the previously described and validated ECC models of CaM-CaMKII-CaN in rabbit ventricular cells (Hund and Rudy, 2004; Grandi et al., 2007; Saucerman and Bers, 2008; Bers and Grandi, 2009; Kraeuter et al., 2010; Soltis and Saucerman, 2010). This will allow testing hypotheses on how the interactions between Ca^{2+}, IP_3, and CaMKII signaling pathways contribute to heart failure phenotypes. Finally, the tools and insights our group develops will be useful to investigate how perturbations in cytosolic and nuclear Ca^{2+} and IP_3signaling affect ECC and ETC in atrial myocytes (Grandi et al., 2011; Koivumäki et al., 2011).

AUTHOR AND CONTRIBUTORS

Designed the work: Anushka P. Michailova and Felix Hohendanner. Performed the experiments: Felix Hohendanner and Lothar A. Blatter. Performed the simulations: Anushka P. Michailova. Analyzed the data: Anushka P. Michailova, Felix

Hohendanner, Lothar A. Blatter, and Andrew D. McCulloch. Contributed reagents/materials/analysis tools: Anushka P. Michailova, Felix Hohendanner, and Lothar A. Blatter. Wrote the paper: Anushka P. Michailova, Felix Hohendanner, Lothar A. Blatter, and Andrew D. McCulloch.

ACKNOWLEDGMENTS

This work is supported by NIBIB 1R03EB014593-01 (Michailova), National Biomedical Computational Resource (NIH grant 8P41 GM103426-19), NHLBI Systems Biology Collaboration 1R01HL105242-01 (McCulloch), and National Institutes of Health grants HL62231, HL80101 and HL101235 and the Leducq Foundation (Blatter).

REFERENCES

Alonso, M. T., and García-Sancho, J. (2011). Nuclear Ca(2+) signalling. *Cell Calcium* 49, 280–289. doi: 10.1016/j.ceca.2010.11.004

Arantes, L. A., Aguiar, C. J., Amaya, M. J., Figueiró, N. C., Andrade, L. M., Rocha-Resende, C., et al. (2012). Nuclear inositol 1,4,5-trisphosphate is a necessary and conserved signal for the induction of both pathological and physiological cardiomyocyte hypertrophy. *J. Mol. Cell. Cardiol.* 53, 475–486. doi: 10.1016/j.yjmcc.2012.06.017

Bare, D. J., Kettlun, C. S., Liang, M., Bers, D. M., and Mignery, G. A. (2005). Cardiac type 2 inositol 1,4,5-triphosphate receptor: interaction and modulation by calcium/calmodulin-dependent protein kinase II. *J. Biol. Chem.* 280, 15912–15920. doi: 10.1074/jbc.M414212200

Berridge, M. J. (2009). Inositol trisphosphate and calcium signaling mechanisms. *Biochem. Biophys. Acta* 1793, 933–940. doi: 10.1016/j.bbamcr.2008.10.005

Bers, D. (2001). *Excitation-Contraction Coupling and Cardiac Contractile Force*. Dortrecht; Boston, MA; London: Kluwer Academic Publishers. doi: 10.1007/978-94-010-0658-3

Bers, D. (2013). Membrane receptor neighborhoods: snuggling up to the nucleus. *Circ. Res.* 112, 224–226. doi: 10.1161/CIRCRESAHA.112.300494

Bers, D. M., and Grandi, E. (2009). CaMKII regulation of cardiac ion channels. *J. Cardiovasc. Pharmacol.* 54, 180–187. doi: 10.1097/FJC.0b013e3181a25078

Bkaily, G., Avedanian, L., Al-Khoury, J., Provost, C., Nader, M., D'Orléans-Juste, P., et al. (2011). Nuclear membrane receptors for ET-1 in cardiovascular function. *Am. J. Physiol. Regul. Integr. Comp. Physiol.*, 300, R251–R263. doi: 10.1152/ajpregu.00736.2009

Bkaily, G., Nader, M., Avedanian, L., Choufani, S., Jacques, D., D'Orléans-Juste, P., et al. (2006). G-protein-coupled receptors, channels, and Na+-H+ exchanger in nuclear membranes of heart, hepatic, vascular endothelial, and smooth muscle cells. *J. Can. Physiol. Pharmacol.* 84, 431–441. doi: 10.1139/y06-002

Blatter, L. A., Kockskamper, J., Sheehan, K. A., Zima, A. V., Huser, J., and Lipsius, S. (2003). Local calcium gradients during excitation-contraction coupling and alternans in atrial myocytes. *J. Physiol.* 546, 19–31. doi: 10.1113/jphysiol.2002.025239

Bootman, M. D., Fearnley, C., Smyrnias, I., MacDonald, F., and Roderick, J. L. (2009). An update on nuclear calcium signaling. *J. Cell Sci.* 122, 2337–2350. doi: 10.1242/jcs.028100

Cooling, M., Hunter, P., and Crampin, E. (2007). Modeling hypertrophic IP3 transients in the cardiac myocyte. *Biophys. J.* 93, 3421–3433. doi: 10.1529/biophysj.107.110031

Crossman, D. J., Ruygrok, P. N., Soeller, C., and Cannell, M. B. (2011). Changes in the organization of excitation-contraction coupling structures in failing human heart. *PLoS ONE* 6:e17901. doi: 10.1371/journal.pone.0017901

Dawson, A. P., Lea, E. J., and Irvine, R. F. (2003). Kinetic model of inositol triphosphate receptor that shows both steady-state and quantal patterns of Ca^{2+} release from intracellular stores. *Biochem. J.* 370, 621–629. doi: 10.1042/BJ20021289

De Young, G. W., and Keizer, J. (1992). A single-pool inositol 1,4,5-triphosphate receptor-based model for agonist-simulated oscillations in Ca^{2+} concentration. *Proc. Natl. Acad. Sci. U.S.A.* 89, 9895–9899. doi: 10.1073/pnas.89.20.9895

Dibb, K. M., Clarke, J. D., Horn, M. A., Richards, M. A., Graham, H. K., Eisner, D. A., et al. (2009). Characterization of an extensive transverse tubular network in sheep atrial myocytes and its depletion in heart failure. *Circ. Heart Fail.* 2, 482–489. doi: 10.1161/CIRCHEARTFAILURE.109.852228

Domeier, T. L., Zima, A. V., Maxwell, J. T., Huke, S., Mignery, G. A., and Blatter, L. A. (2008). IP_3 receptor-dependent Ca^{2+} release modulates excitation-contraction coupling in rabbit ventricular myocytes. *Am. J. Physiol. Heart Circ. Physiol.* 294, H596–H604. doi: 10.1152/ajpheart.01155.2007

Falcke, M. (2003). On the role of stochastic channel behavior in intracellular dynamics. *Biophys. J.* 84, 42–56. doi: 10.1016/S0006-3495(03)74831-0

Fraiman, D., and Dawson, S. P. (2004). A model of the IP_3 receptor with a luminal calcium binding site: stochastic simulations and analysis. *Cell Calcium* 35, 403–413. doi: 10.1016/j.ceca.2003.10.004

Franzini-Armstrong, C., Protasi, F., and Ramesh, V. (1999). Shape, size, and distribution of Ca(2+) release units and couplons in skeletal and cardiac muscles. *Biophys. J.* 77, 1528–1539. doi: 10.1016/S0006-3495(99)77000-1

Genka, C., Ishida, H., Ichimori, K., Hirota, Y., Tanaami, T., and Nakazawa, H. (1999). Visualization of biphasic Ca2+ diffusion from cytosol to nucleus in contracting adult rat cardiac myocytes with an ultra-fast confocal imaging system. *Cell Calcium* 25, 199–208. doi: 10.1054/ceca.1999.0026

Gerasimenko, J. V., Maruyama, Y., Yano, K., Dolman, N. J., Tepikin, A. V., Petersen, O. H., et al. (2003). NAADP mobilizes Ca^{2+} from a thapsigargin-sensitive store in the nuclear envelope by activating ryanodine receptors. *J. Cell Biol.* 163, 271–282. doi: 10.1083/jcb.200306134

Gin, E., Falcke, M., Wagner, L. E. 2nd., Yule, D. I., and Sneyd, J. (2009). A kinetic model of the inositol triphosphate receptor based of single-channel data. *Biophys. J.* 96, 4053–4062. doi: 10.1016/j.bpj.2008.12.3964

Grandi, E., Pandit, S. V., Voigt, N., Workman, A. J., Dobrev, D., Jalife, J., et al. (2011). Human atrial action potential and Ca2+ model: sinus rhythm and chronic atrial fibrillation. *Circ. Res.* 109, 1055–1066. doi: 10.1161/CIRCRESAHA.111.253955

Grandi, E., Puglisi, J. L., Wagner, S., Maier, L. S., Severi, S., and Bers, D. M. (2007). Simulation of Ca-calmodulin-dependent protein kinase II on rabbit ventricular myocyte ion currents and action potential. *Biophys. J.* 93, 3835–3847. doi: 10.1529/biophysj.107.114868

Grynkiewicz, G., Poenie, M., and Tsien, R. Y. (1985). A new generation of Ca2+ indicators with greatly improved fluorescence properties. *J. Biol. Chem.* 260, 3440–3450.

Guatimosim, S., Amaya, M. J., Guerra, M. T., Aguiar, C. J., Goes, A. M., Gómez-Viquez, N. L., et al. (2008). Nuclear Ca2+ regulates cardiomyocyte function. *Cell Calcium* 44, 230–242. doi: 10.1016/j.ceca.2007.11.016

Hake, J. E., Edwards, A. G., Yu, Z., Kekenes-Huskey, P., Michailova, A. P., McCammon, A., et al. (2012). Modeling cardiac calcium sparks in a three-dimensional reconstruction of a calcium release unit. *J. Physiol.* 590, 4403–4422. doi: 10.1113/jphysiol.2012.227926

Harzheim, D., Movassagh, M., Foo, R., Ritter, O., Tashfeen, A., Conway, S. J., et al. (2009). Increased $InsP_3Rs$ in the junctional sarcoplasmic reticulum augment Ca^{2+} transients and arrhythmias associated with cardiac hypertrophy. *Proc. Natl. Acad. Sci. U.S.A.* 106, 11406–11411. doi: 10.1073/pnas.0905485106

He, J. Q., Pi, Y., Walker, J. W., and Kamp, T. J. (2000). Endothelin-1 and photoreleased diacylglycerol increse L-type Ca^{2+} current by activation of protein kinase C in rat ventricular myocytes. *J. Physiol.* 524, 807–820. doi: 10.1111/j.1469-7793.2000.00807.x

Heinzel, F. R., Bito, V., Volders, P. G., Antoons, G., Mubagwa, K., and Sipido, K. R. (2002). Spatial and temporal inhomogeneities during Ca2+ release from the sarcoplasmic reticulum in pig ventricular myocytes. *Circ. Res.* 91, 1023–1030. doi: 10.1161/01.RES.0000045940.67060.DD

Hund, T. J., and Rudy, Y. (2004). Rate dependence and regulation of action potential and calcium transient in a canine cardiac ventricular cell model. *Circulation* 110, 3168–3174. doi: 10.1161/01.CIR.0000147231.69595.D3

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

Ibarra, C. C., Vicencio, J. M., Estrada, M., Lin, Y., Rocco, P., Rebellato, P., et al. (2013). Local control of nuclear calcium signaling in cardiac myocytes by perinuclear microdomains of sarcolemmal insulin-like growth factor 1 receptors. *Circ. Res.* 112, 236–245. doi: 10.1161/CIRCRESAHA.112.273839

Irvine, R. F. (2003). Nuclear lipid signaling. *Nat. Rev. Mol. Cell Biol.* 4, 349–360. doi: 10.1038/nrm1100

Jafri, M. S. (2012). Models of excitation-contraction coupling in cardiac ventricular myocytes. *Methods Mol. Biol.* 910, 309–335. doi: 10.1007/978-1-61779-965-5_14

Jafri, M. S., and Keizer, J. (1994). Diffusion of inositol 1,4,5-triphosphate but not Ca^{2+} is necessary for class of inositol 1,4,5-triphosphate-iduced Ca^{2+} waves. *Proc. Natl. Acad. Sci. U.S.A.* 91, 9485–9489. doi: 10.1073/pnas.91.20.9485

Jafri, M. S., and Keizer, J. (1995). On the roles of Ca^{2+} diffusion, Ca^{2+} buffers, and the endoplasmic reticulum in IP_3-induced Ca^{2+} waves. *Biophys. J.* 69, 2139–2153. doi: 10.1016/S0006-3495(95)80088-3

James, A. F., Ramsey, J. E., Reynolds, A. M., Hendry, B. M., and Shattock, M. J. (2001). Effects of endothelin-1 on K^+ currents from rat ventricular myocytes. *Biochem. Biophys. Res. Commun.* 284, 1048–1055. doi: 10.1006/bbrc.2001.5083

Kim, J. C., Son, M. J., Subedi, K. P., Li, Y., Ahn, J. R., and Woo, S. H. (2010). Atrial local Ca2+ signaling and inositol 1,4,5-trisphosphate receptors. *Prog. Biophys. Mol. Biol.* 103, 59–70. doi: 10.1016/j.pbiomolbio.2010.02.002

Kirk, M. M., Izu, L. T., Chen-Izu, Y., McCulle, S. L., Wier, W. G., Balke, C. W., et al. (2003). Role of transverse-axial tubule system in generating calcium sparks and calcium transients in rat atrial myocytes. *J. Physiol.* 547, 441–451. doi: 10.1113/jphysiol.2002.034355

Kockskämper, 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, 2590–2595. doi: 10.1016/S0006-3495(01)75903-6

Kockskämper, J., Zima, A. V., Roderick, H. L., Pieske, B., Blatter, L. A., and Bootman, M. D. (2008). Emerging roles of inositol 1,4,5-thriphosphate signaling in cardiac myocytes. *J. Mol. Cell. Cardiol.* 45, 128–147. doi: 10.1016/j.yjmcc.2008.05.014

Koivumäki, J. T., Korhonen, T., and Tavi, P. (2011). Impact of sarcoplasmic reticulum calcium release on calcium dynamics and action potential morphology in human atrial myocytes: a computational study. *PLoS Comp. Biol.* 7:e1001067. doi: 10.1371/journal.pcbi.1001067

Kraeuter, M. J., Soltis, A. R., and Saucerman, J. (2010). Modeling cardiac β-adrenergic signaling with normalized-Hill differential equations: comparison with a biochemical model. *BMC Syst. Biol.* 4:157. doi: 10.1186/1752-0509-4-157

Lauer, M. R., Gunn, M. D., and Clusin, W. T. (1992). Endothelin-1 activates voltage-dependent Ca^{2+} current by a G protein-dependent mechanism in rabbit cardiac myocytes. *J. Physiol.* 448, 729–747.

Laurent, M., and Claret, M. (1997). Single-induced Ca^{2+} oscillations through the regulation of the inositol 1,4,5-triphosphate-gared Ca^{2+} channel: an allosteric model. *J. Theor. Biol.* 186, 307–326. doi: 10.1006/jtbi.1996.0365

LeBeau, A. P., Yule, D. I., Groblewski, G. E., and Sneyd, J. (1999). Agonist-dependent phosphorylation of 1,4,5-triphosphate receptor: a possible mechanism for agonist-specific calcium oscillations in pancreatic acinar cells. *J. Gen. Physiol.* 113, 851–872. doi: 10.1085/jgp.113.6.851

Ledeen, R., and Wu, G. (2007). GM1 in the nuclear envelope regulates nuclear calcium through association with a nuclear sodium-calcium exchanger. *J. Neurochem. Suppl.* 1, 126–134. doi: 10.1111/j.1471-4159.2007.04722.x

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(IP_3)-receptor type 2-deficient mice. *Circ. Res.* 96, 1274–1281. doi: 10.1161/01.RES.0000172556.05576.4c

Li, Y. X., and Rinzel, J. (1994). Equations for $InsP_3$ receptor-mediated $[Ca^{2+}]_i$ oscillations derived from a detailed kinetic model: a Hodgkin-Huxley like formalism. *J. Theor. Biol.* 166, 461–473. doi: 10.1006/jtbi.1994.1041

Ljubojevic, S., Walther, S., Asgarzoei, M., Sedej, S., Pieske, B., and Kockskämper, J. (2011). *In situ* calibration of nucleoplasmic versus cytoplasmic Ca^{2+} concentration in adult cardiomyocytes. *Biophys. J.* 100, 2356–2366. doi: 10.1016/j.bpj.2011.03.060

Louch, W. E., Bito, V., Heinzel, F. R., Macianskiene, R., Vanhaecke, J., Flameng, W., et al. (2004). Reduced synchrony of Ca2+ release with loss of T-tubules-a comparison to Ca2+ release in human failing cardiomyocytes. *Cardiovasc. Res.* 62, 63–73. doi: 10.1016/j.cardiores.2003.12.031

Mackenzie, L., Roderick, H. I., Proven, A., Conway, S. J., and Bootman, M. D. (2004). Inositol 1,4,5,-triphosphate receptors in the heart. *Biol. Res.* 37, 553–557. doi: 10.4067/S0716-97602004000400008

Mak, D., McBride, S., and Foskett, J. K. (2001). Regulation by Ca^{2+} and inositol 1,4,5-triphosphate ($InsP_3$) of single recombinant type 3 $InsP_3$ receptor channels: Ca^{2+} activation uniquely distinguishes types 1 and 3 $InsP_3$ receptors. *J. Gen. Physiol.* 117, 435–446. doi: 10.1085/jgp.117.5.435

Malviya, A. N., and Klein, C. (2006). Mechanism regulating nuclear calcium signaling. *Can. J. Physiol. Pharmacol.* 84, 403–422. doi: 10.1139/y05-130

Maxwell, J. T., and Blatter, L. A. (2012). Facilitation of cytosolic calcium wave propagation by local calcium uptake into the sarcoplasmic reticulum in cardiac myocytes. *J. Physiol.* 590, 6037–6045. doi: 10.1113/jphysiol.2012.239434

Means, S., Smith, A. J., Shepherd, J., Shadid, J., Fowler, J., Wojcikiewicz, R. H. J., et al. (2006). Reaction diffufion modeling of calcium dynamics with realistic ER geometry. *Biophys. J.* 91, 537–557. doi: 10.1529/biophysj.105.075036

Michell, R. H., Kirk, C. J., Jones, L. M., Downes, C. P., and Creba, J. A. (1981). The stimulation of inositol lipid metabolism that accompanies calcium mobilization in stimulated cells: defined characteristics and unanswered questions. *Philos. Trans. R. Soc. Lond. Biol. Sci.* 296, 123–138. doi: 10.1098/rstb.1981.0177

Molkentin, J. D., Lu, J. R., Antos, C. L., Markham, B., Richardson, J., Robbins, J., et al. (1998). A calcineurin-dependent transcriptional pathway for cardiac hypertrophy. *Cell* 93, 215–228. doi: 10.1016/S0092-8674(00)81573-1

Moraru, I., Kaftan, E. J., Ehrlich, B. E., and Watras, J. (1999). Regulation of type 1 inositol 1,4,5-triphosphate-gated calcium channels by $InsP_3$ and calcium: simulation of single channel kinetics based on ligand binding and electro physiological analysis. *J. Gen. Physiol.* 113, 837–849. doi: 10.1085/jgp.113.6.837

Nakayama, H., Bodi, I., Maillet, M., DeSantiago, J., Domeier, T. L., Mikoshiba, K., et al. (2010). The IP_3 receptor regulates cardiac hypertrophy in response to select stimuli. *Circ. Res.* 107, 659–666. doi: 10.1161/CIRCRESAHA.110.220038

Noble, D. (2011). Successes and failures in modeling heart cell electrophysiology. *Heart Rhythm* 8, 1798–1803. doi: 10.1016/j.hrthm.2011.06.014

Noble, D., Garny, A., and Noble, P. J. (2012). How the Hodgkin-Huxley equations inspired the cardiac physiome project. *J. Physiol.* 590, 2613–2628. doi: 10.1113/jphysiol.2011.224238

Petersen, O. H., Petersen, C. C., and Kasai, H. (1994). Calcium and hormone action. *Annu. Rev. Physiol.* 56, 297–319. doi: 10.1146/annurev.ph.56.030194.001501

Poláková, E., and Sobie, E. A. (2013). Alterations in T-tubule and dyad structure in heart disease: challenges and opportunities for computational analyses. *Cardiovasc. Res.* 98, 233–239. doi: 10.1093/cvr/cvt026

Proven, A., Roderick, H. L., Conway, S. J., Berridge, M. J., Horton, J. K., Capper, S. J., et al. (2006). Inositol 1,4,5,-supports the arrhythmogenic action of endothelin-1 on ventricular cardiac myocytes. *J. Cell Sci.* 119, 3363–3375. doi: 10.1242/jcs.03073

Puglisi, J. L., Yuan, W., Timofeyev, V., Myers, R. E., Chiamvimonvat, N., Samarel, A. M., et al. (2011). Phorbol ester and endothelin-1 alter functional expression of Na+/Ca2+ exchange, K+, and Ca2+ currents in cultured neonatal rat myocytes. *Am. J. Physiol. Heart Circ. Physiol.* 300, H617–H626. doi: 10.1152/ajpheart.00388.2010

Remus, T. P., Zima, A. V., Bossuyt, J., Bare, D. J., Martin, J. L., and Blatter, L. A. (2006). Biosensors to measure inositol 1,4,5-triphosphate concentration in living cells with spatiotemporal resolution. *J. Biol. Chem.* 281, 608–616. doi: 10.1074/jbc.M509645200

Richards, M. A., Clarke, J. D., Saravanan, P., Voigt, N., Dobrev, D., Eisner, D. A., et al. (2011). Transverse tubules are a common feature in large mammalian atrial myocytes including human. *Am. J. Physiol. Heart Circ. Physiol.* 301, H1996–H2005. doi: 10.1152/ajpheart.00284.2011

Roderick, H. L., and Bootman, M. D. (2003). Bi-directional signaling from the InsP3 receptor: regulation by calcium and accessory factors. *Biochem. Soc. Trans.* 31, 950–953. doi: 10.1042/BST0310950

Saucerman, J., and Bers, D. M. (2008). Calmodulin mediates differential sensitivity of CaMKII and calcineurin to local Ca^{2+} in cardiac myocytes. *Biophys. J.* 95, 4597–4612. doi: 10.1529/biophysj.108.128728

Shannon, T., Wang, F., Puglisi, J., Weber, C. H. R., and Bers, D. B. (2004). A mathematical treatment of integrated Ca dynamic within the ventricular myocyte. *Biophys. J.* 87, 3351–3371. doi: 10.1529/biophysj.104.047449

Shkryl, V. M., Maxwell, J. T., and Blatter, L. A. (2012). A novel method for spatially complex diffraction-limited photoactivation and photobleaching in living cells. *J. Physiol.* 590, 1093–1100. doi: 10.1113/jphysiol.2011.223446

Shuai, J. W., and Jung, P. (2002). Stochastic properties of Ca^{2+} release of inositol 1,4,5-trisphosphate receptor clusters. *Biophys. J.* 83, 87–97. doi: 10.1016/S0006-3495(02)75151-5

Siekmann, I., Wagner, L. E. 2nd., Yule, D., Crampin, E. J., and Sneyd, J. (2012). A kinetic model for type I and II IP3R accounting for mode changes. *Biophys. J.* 103, 658–668. doi: 10.1016/j.bpj.2012.07.016

Signore, S., Sorrentino, A., Ferreira-Martins, J., Kannappan, R., Shafaie, M., Del Ben, F., et al. (2013). Inositol 1, 4, 5-trisphosphate receptors and human left ventricular myocytes. *Circulation* 128, 1286–1297. doi: 10.1161/CIRCULATIONAHA.113.002764

Smith, I. F., Wiltgen, S. M., and Parker, I. (2009). Localization of puff sites adjacent to the plasma membrane: functional and spatial characterization of Ca2+ signaling in SH-SY5Y cells utilizing membrane-permeant caged IP3. *Cell Calcium* 45, 65–76. doi: 10.1016/j.ceca.2008.06.001

Sneyd, J., and Dufour, J. F. (2002). A dynamic model of the type-2 inositol triphosphate receptor. *Proc. Natl. Acad. Sci. U.S.A.* 99, 2398–2403. doi: 10.1073/pnas.032281999

Sobie, E. A., and Lederer, W. J. (2012). Dynamic local changes in sarcoplasmic reticulum calcium: physiological and pathophysiological roles. *J. Mol. Cell. Cardiol.* 52, 304–311. doi: 10.1016/j.yjmcc.2011.06.024

Soltis, A. R., and Saucerman, J. (2010). Synergy between CaMKII substrates and β-adrenergic signaling in regulation of cardiac myocyte Ca^{2+} handling. *Biophys. J.* 99, 2038–2047. doi: 10.1016/j.bpj.2010.08.016

Swillens, S., Champeil, P., Combettes, L., and Dupont, G. (1998). Stochastic simulation of a single inositol 1,4,5-trisphosphate-sensitive Ca2+ channel reveals repetitive openings during 'blip-like' Ca2+ transients. *Cell Calcium* 23, 291–302. doi: 10.1016/S0143-4160(98)90025-2

Tadevosyan, A., Vaniotis, G., Allen, B. G., Hebert, T. E., and Nattel, S. (2012). G protein-coupled receptor signalling in the cardiac nuclear membrane: evidence and possible roles in physiological and pathophysiological function. *J. Physiol.* 590, 1313–1330. doi: 10.1113/jphysiol.2011.222794

Takahashi, A., Camacho, P., Lechleiter, J. D., and Herman, B. (1999). Measurement of intracellular calcium. *Physiol. Rev.* 79, 1089–1125.

Thul, R., and Falcke, M. (2004). Release currents of IP_3 receptor channel clusters and concentration profiles. *Biophys. J.* 86, 2660–2673. doi: 10.1016/S0006-3495(04)74322-2

Vaniotis, G., Allen, B. G., and Hebert, T. E. (2011). Nuclear GPCRs in cardiomyocytes: an insider's view of beta-adrenergic receptor signaling. *Am. J. Physiol. Heart Circ. Physiol.* 301, H1754–H1764. doi: 10.1152/ajpheart.00657.2011

Vogelsand, M., Broede-Sitz, A., Schafer, E., Zerkowski, H. R., and Brodde, O. E. (1994). Endothelin ET_A-receptors couple to inositol phosphate formation and inhibition of adenylate cyclase in human right atrium. *J. Cardiovasc. Pharmacol.* 23, 344–347. doi: 10.1097/00005344-199402000-00025

Wang, H., and Clapham, D. E. (1999). Conformational changes of the *in situ* nuclear pore complex. *Biophys. J.* 77, 241–247. doi: 10.1016/S0006-3495(99)76885-2

Watanabe, T., and Endoh, M. (1999). Characterization of the endothelin-1 induced regulation of L-type Ca^{2+} current in rabbit ventricular myocytes. *Naunyn Schmiedebergs Arch. Pharmacol.* 360, 654–664. doi: 10.1007/s002109900130

Wilhelms, M., Hettmann, H., Maleckar, M. M., Koivumäki, J. T., Dössel, O., and Seemann, G. (2013). Benchmarking electrophysiological models of human atrial myocytes. *Front. Physiol.* 3:487. doi: 10.3389/fphys.2012.00487

Woo, S. H., and Lee, C. (1999). Effects of endothelin-1 on Ca^{2+} signaling in guinea-pig ventricular myocytes: role of protein kinase C. *J. Mol. Cell. Cardiol.* 31, 613–643. doi: 10.1006/jmcc.1998.0899

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. doi: 10.1016/j.pharmthera.2005.04.002

Wu, G., Xie, X., Lu, Z. H., and Ledeen, R. W. (2009). Sodium-calcium exchanger complexed with GM1 ganglioside in nuclear membrane transfers calcium from nucleoplasm to endoplasmic reticulum. *Proc. Natl. Acad. Sci. U.S.A.* 106, 10829–10834. doi: 10.1073/pnas.0903408106

Wu, X., Zhang, T., Bossuyt, J., Li, X., McKinsey, T. A., Dedman, J. R., et al. (2006). Local $InsP_3$-dependent perinuclear Ca^{2+} signaling in cardiac myocyte excitation-transcription coupling. *J. Clin. Invest.* 116, 657–682. doi: 10.1172/JCI27374

Yang, H. T., Sakurai, K., Sugawara, H., Watanabe, T., Norota, I., and Endoh. M. (1999). Role of Na^+/Ca^{2+} exchange in endothelin-1-induced increases in Ca^{2+} transient and contractility in rabbit ventricular myocytes: pharmacological analysis with KB-R7943. *Br. J. Pharmacol.* 126, 1785–1795. doi: 10.1038/sj.bjp.0702454

Youm, J. B., Kim, N., Han, J., Kim, E., Joo, H., Leem, C. H., et al. (2006). A mathematical model of pacemaker activity recorded from mouse small intestine. *Philos. Trans. A Math. Phys. Eng. Sci.* 364, 1135–1154. doi: 10.1098/rsta.2006.1759

Zhang, Y. H., James, A. F., and Hancox, J. C. (2001). Regulation by endothelin-1 of Na^+-Ca^{2+} exchange current (I_{NaCa}) from guinea-pig isolated ventricular myocytes. *Cell Calcium* 30, 351–360. doi: 10.1054/ceca.2001.0244

Zima, A. V., Bare, D. J., Mignery, G. A., and Blatter, L. A. (2007). IP_3-dependent nuclear Ca^{2+} signaling in the mammalian heart. *J. Physiol.* 584, 601–611. doi: 10.1113/jphysiol.2007.140731

Zima, A. V., and Blatter, L. A. (2004). Inositol 1,4,5-triphosphate-dependent Ca^{2+} signaling in cat atrial excitation-contraction coupling and arrhythmias. *J. Physiol.* 555, 607–615. doi: 10.1113/jphysiol.2003.058529

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Received: 18 December 2013; accepted: 18 February 2014; published online: 06 March 2014.

*Citation: Hohendanner F, McCulloch AD, Blatter LA and Michailova AP (2014) Calcium and IP3 dynamics in cardiac myocytes: experimental and computational perspectives and approaches. Front. Pharmacol. **5**:35. doi: 10.3389/fphar.2014.00035*

This article was submitted to Pharmacology of Ion Channels and Channelopathies, a section of the journal Frontiers in Pharmacology.