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Journal of Molecular and Cellular Cardiology 45 (2008) 128–147
Journal of Molecular and Cellular Cardiology
www.elsevier.com/locate/yjmcc

Review article

Emerging roles of inositol 1,4,5-trisphosphate signaling in cardiac myocytes

Jens Kockskämper [a,*], Aleksey V. Zima [b], H. Llewelyn Roderick [c,d], Burkert Pieske [a], Lothar A. Blatter [b], Martin D. Bootman [c]

[a] *Division of Cardiology, Medical University of Graz,, Auenbruggerplatz 15, A-8036 Graz, Austria*
[b] *Department of Molecular Biophysics & Physiology, Rush University, 1750 W. Harrison St., Chicago, IL 60612, USA*
[c] *Laboratory of Molecular Signalling, Babraham Institute, Cambridge CB2 4AT, UK*
[d] *Department of Pharmacology, University of Cambridge, Tennis Court Road, Cambridge CB2 1 PD, UK*

Received 3 April 2008; received in revised form 20 May 2008; accepted 21 May 2008
Available online 15 June 2008

Abstract

Inositol 1,4,5-trisphosphate (IP_3) is a ubiquitous intracellular messenger regulating diverse functions in almost all mammalian cell types. It is generated by membrane receptors that couple to phospholipase C (PLC), an enzyme which liberates IP_3 from phosphatidylinositol 4,5-bisphosphate (PIP_2). The major action of IP_3, which is hydrophilic and thus translocates from the membrane into the cytoplasm, is to induce Ca^{2+} release from endogenous stores through IP_3 receptors (IP_3Rs). Cardiac excitation–contraction coupling relies largely on ryanodine receptor (RyR)-induced Ca^{2+} release from the sarcoplasmic reticulum. Myocytes express a significantly larger number of RyRs compared to IP_3Rs (~100:1), and furthermore they experience substantial fluxes of Ca^{2+} with each heartbeat. Therefore, the role of IP_3 and IP_3-mediated Ca^{2+} signaling in cardiac myocytes has long been enigmatic. Recent evidence, however, indicates that despite their paucity cardiac IP_3Rs may play crucial roles in regulating diverse cardiac functions. Strategic localization of IP_3Rs in cytoplasmic compartments and the nucleus enables them to participate in subsarcolemmal, bulk cytoplasmic and nuclear Ca^{2+} signaling in embryonic stem cell-derived and neonatal cardiomyocytes, and in adult cardiac myocytes from the atria and ventricles. Intriguingly, expression of both IP_3Rs and membrane receptors that couple to PLC/IP_3 signaling is altered in cardiac disease such as atrial fibrillation or heart failure, suggesting the involvement of IP_3 signaling in the pathology of these diseases. Thus, IP_3 exerts important physiological and pathological functions in the heart, ranging from the regulation of pacemaking, excitation–contraction and excitation–transcription coupling to the initiation and/or progression of arrhythmias, hypertrophy and heart failure.

Keywords: Inositol 1,4,5-trisphosphate; Cardiac myocyte; Calcium; Inotropy; Arrhythmias; Nucleus; Hypertrophy

Contents

* Corresponding author.
E-mail address: jens.kockskaemper@meduni-graz.at (J. Kockskämper).

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doi:10.1016/j.yjmcc.2008.05.014

1. The discovery of IP_3

A quarter of a century ago it was shown that D-*myo* inositol 1,4,5-trisphosphate (IP_3) releases Ca^{2+} from a non-mitochondrial internal Ca^{2+} store [1]. Since this hallmark discovery, IP_3 has emerged as a ubiquitous intracellular messenger, releasing Ca^{2+} from stores through activation of IP_3 receptors (IP_3Rs) in almost all eukaryotic cells. The major IP_3-sensitive intracellular Ca^{2+} store is the endoplasmic reticulum. However, IP_3 has also been shown to release Ca^{2+} stored in other compartments, such as the Golgi and the nuclear envelope [2]. In addition, IP_3Rs are present on the plasma membrane of some cell types, where they can gate Ca^{2+} influx [3]. A crucial role for IP_3-dependent Ca^{2+} release has been demonstrated in many mammalian cell types, ranging from tiny platelets, where it initiates blood clotting, to the impressive dendritic trees of cerebellar Purkinje neurons, where it is involved in the regulation of motor function. In the cardiovascular system, IP_3-induced Ca^{2+} release from the sarcoplasmic reticulum (SR) plays a key role in pharmaco-mechanical coupling in smooth muscle cells of the vasculature, and thus in the regulation of peripheral resistance and blood pressure. Despite being recognized as a potential messenger in cardiac myocytes nearly two decades ago, the role of IP_3 has been enigmatic. However, a number of recent reports have begun to unravel the physiological and potentially pathological actions of IP_3 within the heart.

2. The ABC of IP_3: where does it come from and where does it go?

IP_3 is generated by hydrolysis of phosphatidylinositol 4,5-bisphosphate (PIP_2) through phosphoinositide-specific phospholipase C (PLC). PIP_2 is a relatively minor phospholipid (~1% of total anionic phospholipids), but it is the main polyphosphoinositide in the sarcolemma. Its concentration in myocardium is in the range of 10–30 μM or 150–450 pmol/mg protein [4,5]. PIP_2 itself serves important signaling functions, including the regulation of ion channels and transporters and the anchoring of cytoskeletal proteins at the membrane [6,7]. Furthermore, it is the precursor of phosphatidylinositol 3,4,5-trisphosphate (PIP_3), a phosphoinositide involved in cell signaling. There are at least 13 phosphoinositide-specific PLC isoforms grouped into six subfamilies: β, γ, δ, ε, η and ζ [8,9]. Members of the β, γ, δ and ε subfamilies are expressed in cardiac myocytes. PLCs may be activated by heptahelical G protein-coupled receptors (PLCβ), receptor tyrosine kinases (PLCγ), PIP_2 and Ca^{2+} (PLCδ) or Ras (PLCε). Consequently, many transmitters, neurohormonal factors, hormones and other stimuli (e.g. stretch) may increase IP_3 concentration and activate IP_3-induced Ca^{2+} release in cardiac myocytes downstream of PLC activation. Upon stimulation, intracellular IP_3, or inositol phosphate concentration in general, has been shown to increase by a factor of >12 [10]. However, it should be pointed out that most of these data were determined from assays of measuring total inositol phosphate accumulation over time (in the presence of the inositol monophosphatase inhibitor Li^+). Such measurements do not reflect the *in vivo* steady-state IP_3 increase, which is likely to be quite modest. Recently, the concentration of free IP_3 was estimated directly using a novel FRET-based biosensor. Following maximal stimulation of α-adrenergic and endothelin receptors, free IP_3 in cardiac myocytes increased to ~30 nM [11]. While such biosensors are useful for detecting genuine increases in IP_3 concentration, they are less helpful in revealing the kinetics of IP_3 turnover, since binding of IP_3 to the probe buffers the molecule and protects it from hydrolyzing enzymes. Based on modeling studies, it was estimated that stimulation of atrial myocytes with endothelin transiently increases IP_3 concentration from a basal value of ~15 nM to a maximal value of ~35 nM within ~400 s, after which the IP_3 level gradually declines and returns to baseline within tens of minutes [12]. A note of caution as to the quantitative results obtained with isolated myocytes is warranted though, since careful studies indicate that PIP_2 and inositol phosphate levels drop dramatically during the isolation procedure [13,14].

Because IP_3 is hydrophilic, it translocates from the sarcolemma to the cytoplasm upon formation from PIP_2. The main cytoplasmic target of IP_3 is IP_3Rs in the membrane of the ER/SR. There are three IP_3R isoforms, denoted type 1, type 2 and type 3. Current evidence suggests that the heart expresses all three IP_3R isoforms, although there is some inconsistency in the literature regarding their relative ratios in different cardiac cells. It has been proposed that type 1 IP_3Rs are dominant in human atrial and rat Purkinje myocytes [15,16], whereas atrial and ventricular myocytes from most other animal species express predominantly type 2 IP_3R and, to a lesser extent, type 3 IP_3R (e.g. [17,18]). Overall, the majority of studies have concluded that the most prevalent IP_3R isoform within contractile cardiomyocytes is type 2 (see Table 1). The few reports describing

Table 1
Expression of IP3R isoforms in adult myocardium

Species	IP_3R isoform	Specimen	Methods	Confirmed in myocytes?	Findings	Reference
Bovine	1	A, V, CS	WB, IHC, IP_3 binding	Yes	IP_3R1: P>AV>>A, V	[16]
Canine	1, 2	LA tissue, myocytes	RT-PCR, WB, IHC	Yes	IP_3R1 in cyto; IP_3R2 in cyto, NE, ID; $IP_3R1,2$ up in AF	[170]
Ferret, Rat	1, 2, 3	Heart, V myocytes	RNA analyses, IP_3 binding, IP_3R reconstitution	Yes ($IP_3R2,3$)	IP_3R1 predominant in myocardium, but absent in myocytes; $IP_3R2,3$ predominant in myocytes (85% vs. 14%)	[57]
Human	1	LV, RV, Septum	NB, *in situ* hybridization	Yes	IP_3R1 up in HF	[19]
Human	1	RA tissue	RT-PCR, WB, IHC	Yes	IP_3R1 in cyto, NE; IP_3R1 up in AF	[15]
Human	1	RA tissue	RT-PCR	No	IP_3R1 up in AF	[169]
Human	1, 2, 3	RA tissue	RT-PCR	No	$IP_3R1,2,3$ down in diabetes	[184]
Mouse	n.d.	Heart; V myocytes	WB, ICC	Yes	Striated pattern in cyto	[142]
Mouse	n.d.	Heart; V myocytes	WB, ICC	Yes	Striated pattern in cyto; colocalization with NCX	[179]
Mouse	1, 2	Heart	WB	No	No changes in diabetes	[183]
Mouse	1, 2, 3	A, V	WB	No	$IP_3R2 \gg IP_3R1,3$; IP_3R2: A>V	[17]
Rabbit	2	LV myocytes	WB	Yes	IP_3R2 up in HF	[202]
Rabbit	2, 3	A, V myocytes	WB	Yes	$IP_3R2,3$: A>V	[41]
Rat	n.d.	Heart, A, V	WB, IHC, EM	Yes	IP_3R in ID in A, V	[203]
Rat	n.d.	Heart, A, V	RNA analyses, WB, IHC, *in situ* hybridization	Yes	IP_3R expression in cardiomyocytes ($RyR:IP_3R \approx 50:1$)	[54]
Rat	n.d.	Heart, A, V, CS	RNA analyses, *in situ* hybridization	Yes	IP_3R: CS>A=V; Senescence: A>V	[204]
Rat	1, 2, 3	A, V, Purkinje	RT-PCR, WB, ICC, IP_3 binding	Yes (IP_3R2)	A>V; IP_3R2 in A myocytes in subsarcolemmal SR	[58]
Rat	2	A myocytes	ICC	Yes	IP_3R2 colocalizes with RyR in subsarcolemmal SR	[63]
Rat	1, 2	A, V	RT-PCR WB	No	$IP_3R1,2$: LA>RA≫LV=RV; $IP_3R1,2$ up by stress in glucocorticoid-dependent manner	[138]
Rat	2	Heart (nuclear fraction), V myocytes	WB, ICC	Yes	IP_3R2 predominant in NE	[18]
Rat	1	LA, LV	RT-PCR, WB	No	IP_3R1 down after depletion of catecholamines	[205]
Rat	2	Heart	RT-PCR, WB	No	IP_3R2 down in diabetes	[185]
Rat	1, 2, 3	LA, LV, RA, RV	RT-PCR, WB	No	$IP_3R1,2$ up with aging	[168]

Abbreviations: A, atrium; AF, atrial fibrillation; AV, atrio-ventricular; CS, conduction system; cyto, cytoplasm; EM, electron microscopy; HF, heart failure; ICC, immunocytochemistry; ID, intercalated discs; IHC, immunohistochemistry; L, left; NCX, Na^+/Ca^{2+} exchanger; NB, Northern blotting; n.d., not determined; NE, nuclear envelope; P, Purkinje; R, right; RT-PCR, reverse transcription or real time polymerase chain reaction; V, ventricle; WB, Western blotting.

type 1 IP_3Rs being more abundant [15,16,19] could indicate differences in IP_3R expression between species, or that the probes employed lacked specificity.

The IP_3R isoforms vary with respect to IP_3 affinity, activation and inactivation by Ca^{2+}, regulation by ATP, phosphorylation and other modulatory factors (e.g. [20,21]). It is believed that subtle variations in the sensitivity of the three IP_3R isoforms to such allosteric regulators can modulate the biophysical properties of the channels. Thus, depending on the expression and subcellular distribution of particular IP_3R isoforms, cells may respond to alterations in IP_3, Ca^{2+} and other modulators quite differently. Furthermore, IP_3Rs form macromolecular signaling complexes with other proteins including ankyrin B, chromogranin A and B, cytochrome *c*, ERp44, Homer, IP_3R-binding protein released with IP_3 (IRBIT), the Na^+/K^+ pump, protein phosphatases 1 and 2A, receptor for activated C kinase 1 (RACK1) and others (for reviews, see [22–24]). The interaction of specific accessory proteins has been demonstrated to either positively or negatively regulate IP_3R opening, with consequent alteration of cellular Ca^{2+} signals (e.g. [25,26]). Although most of the data exploring IP_3R-binding proteins has been derived from studies of neuronal IP_3Rs, it is likely that cardiac IP_3Rs form similar macromolecular complexes. As yet, only Ca^{2+}- and calmodulin-dependent protein kinase II (CaMKII) has been identified as an accessory protein of cardiac IP_3Rs [18].

An intriguing aspect of IP_3 signaling is the presence of the entire phosphoinositide signaling network in the nucleus [27]. In addition, many receptors that act as signal transducers at the cell membrane are also found in the nuclear envelope (e.g. ET_A and ET_B receptors [28]) where they might activate a nuclear PIP_2-PLC-IP_3 cascade. The mechanism for stimulation of nuclear hormone receptors is not entirely clear, although it has been suggested that activated receptors can be internalized from the surface of cells and translocated to the nucleus where they engage signaling pathways. It is therefore plausible that nuclei may locally generate their own IP_3. Alternatively, given that IP_3

is highly diffusible inside cells [29], the IP_3 produced at the plasma membrane may be sufficient for activation of IP_3Rs throughout a cell. The particular interest in cardiac nuclear IP_3 signaling derives from a number of recent studies that have demonstrated expression of IP_3Rs within or around the nucleus in cardiac myocytes [18], and involvement of perinuclear Ca^{2+} signals in the regulation of kinases (e.g. nuclear CaMKII), transcription and nuclear pores. This concept is discussed in more detail below.

It is important to realize that the PIP_2-PLC-IP_3 cascade is just a small part of a much larger signaling network, the inositide metabolome, which encompasses more than 80 gene products involved in the regulation of phosphatidylinositol phosphates and inositol phosphates. The inositide metabolome includes lipid kinases and phosphatases, lipases, inositol phosphate kinases and phosphatases (reviewed in [30]). Inositol polyphosphates derived from IP_3 may be signaling molecules in their own right. In particular, IP_4, IP_5 and IP_6 have been implicated in the regulation of nuclear functions (gene expression, chromatin remodeling, mRNA export and RNA editing). This adds to the complexity of IP_3 signaling and suggests that the potential roles of IP_3 go beyond the release of Ca^{2+} from internal stores. Higher inositol polyphosphates, including IP_4, IP_5 and IP_6, have been detected in the heart [31], as well as high affinity binding sites for IP_4 and IP_6 [32,33]. However, their physiological functions in cardiac myocytes remain poorly understood.

3. Tools to study cardiac IP_3 signaling

Numerous tools are available to study cellular IP_3 signaling. They can be grouped into (A) methods to measure intracellular IP_3 levels, (B) pharmacological tools to manipulate IP_3 levels, IP_3 production and degradation, and the IP_3 receptor, and most recently (C) approaches that involve genetic manipulation of the intracellular IP_3 signaling cascade and the generation of transgenic animals with an altered IP_3 second messenger system.

(A) Of great interest for many years has been the quantitative determination of intracellular IP_3 levels. Until very recently, the real-time study of IP_3 formation, concentration dynamics and spatial distribution at the level of single living cells has not been possible. Instead, measurements have relied upon destructive methodologies [34–37]. These assays essentially rely on whole cell extracts for mass analysis by competition binding, gas chromatography/mass spectrometry and ion exchange chromatography, or metabolic measurements of radio-labeled IP_3 precursors and degradation products. While these methods have generated important insights into cellular IP_3 dynamics, they are rather limited in deciphering the spatiotemporal organization of the IP_3 second messenger system at the cellular and subcellular level. Recent developments, however, have seen the arrival of novel fluorescent probes to study intracellular IP_3 dynamics. For example, a biosensor derived from the IP_3 binding domain of type-3 IP_3R, termed 'LIBRA', was employed to measure IP_3 concentrations in living cells [38]. Similarly, the intracellular translocation of a pleckstrin homology domain from phospholipase $C_{\partial 1}$ fused to GFP was used to estimate $[IP_3]$ [39], and to measure agonist-induced oscillatory changes of IP_3 concentration [40]. A recent study reported the construction and characterization of prototypic fluorescent biosensors that allow quantitative measurements of cellular IP_3 levels in a living cell with temporal and spatial resolution [11]. These sensors (termed FIRE-1 and -3, for 'Fluorescent IP_3 Responsive Element' type-1 and -3) utilized the IP_3R type-1 and type-3 ligand-binding domains expressed as chimeras terminally linked to CFP and YFP, and which could be used as FRET indicators of IP_3. These constructs have been successfully employed to measure $[IP_3]$ in neonatal and adult ventricular myocytes with high temporal and subcellular spatial resolution. For example, in FIRE-1-expressing ventricular myocytes, the agonists endothelin-1, phenylephrine and angiotensin II all produced rapid and spatially resolved increases of IP_3 concentration, with free IP_3 levels rising to 30 nM. A point source of IP_3, experimentally achieved by membrane rupture with a patch pipette containing IP_3, allowed detailed spatiotemporal monitoring of intracellular IP_3 diffusion, revealing that IP_3 diffusion into the nucleus occurred with a delay, and that the elevation of nuclear IP_3 concentration had a blunted rise compared to that in the cytoplasm. Furthermore, stimulation with endothelin-1 (100 nM) caused $[IP_3]$ to rise more rapidly and to higher levels in the cytoplasm as compared with the nucleus. Average data indicate that the amplitude of rise in nuclear $[IP_3]$ was $70\pm9\%$ of that in the cytoplasm, and the $t_{1/2}$ was about twice as long (1.3 min vs. 0.7 min) [11]. Clearly, these new biosensors are exciting and promising novel tools to study IP_3 dynamics at high temporal and spatial resolution in intact living cells, although their potential to buffer IP_3 has to be taken into account.

(B) More conventional, but nonetheless invaluable tools for the study of IP_3 signaling are pharmacological agents that interfere with the IP_3 signaling cascade. Some commonly used reagents are blockers of IP_3 formation (e.g. the PLC inhibitor U73122 and its negative control U73343), or antagonists of the IP_3 receptor (e.g. heparin, 2 aminoethoxydiphenyl borate (2-APB), xestospongins, curcumin). Like many pharmacological inhibitors, these tools all suffer from variable degrees of specificity, have numerous intracellular targets and unwanted side effects or lack membrane permeability (for discussion see [41–43]). Recently developed membrane-permeable forms of IP_3R agonists [44] and IP_3 species that can be activated photolytically [45,46] have further expanded the repertoire of tools for studying IP_3 signaling.

(C) Molecular technologies are also beginning to be employed to explore cardiac IP_3 function. In particular, the generation of animals or cells with genetically altered IP_3 signaling pathways, and the development of transgenic animals. Due to the inherent nature of the rapid developments in this field, we will limit our review to two exemplary reports. The adenoviral-mediated expression of an IP_3 affinity trap (which consisted of the ligand-binding domain of the rat type-1 IP_3R; also known as an 'IP_3 sponge') has been successfully used to abolish IP_3-evoked effects on Ca^{2+} signaling in rabbit ventricular myocytes [41]. The IP_3 sponge represents an experimental tool that can complement or replace pharmacological IP_3R blockers, and specifically attenuate IP_3 signaling. Potentially even better than the IP_3 sponge would be the

introduction of an IP_3 metabolizing enzyme (e.g. the IP_3 5' phosphatase), which does not necessarily have to be present at 1:1 stoichiometry to completely clamp IP_3 signaling. With respect to transgenic animals, it has been shown that the positive inotropic and pro-arrhythmic effects of endothelin-1 were completely abolished in mouse atrial cells lacking the IP_3R type-2 isoform [17]. This loss of response to endothlelin-1 was specifically coupled to the absence of IP_3Rs, since the myocytes retained a normal positive inotropic response to β-adrenergic stimulation.

4. The dilemma of cardiac IP_3 receptors: lost in an ocean of ryanodine receptors

The evidence for IP_3R expression in the heart is indisputable. Numerous laboratories using a variety of techniques have established that cardiac myocytes possess these Ca^{2+} channels. Indeed, the notion that IP_3 mediates Ca^{2+} release in the heart is not new, and has been established by studies spanning the last two decades. The very earliest studies on cardiac IP_3 signaling found evidence for functional IP_3Rs capable of releasing Ca^{2+} from the SR [47–51]. However, compared with Ca^{2+}-induced Ca^{2+} release through RyRs, though, the IP_3-induced Ca^{2+} release was slow and small. These data indicated that a major contribution of IP_3-induced Ca^{2+} release to cardiac excitation–contraction coupling was highly unlikely. As a result, IP_3Rs have been largely overlooked, and often regarded as making a minor contribution, if any, to cardiac Ca^{2+} signaling.

Cardiac excitation–contraction coupling relies principally on Ca^{2+}-induced Ca^{2+} release through type 2 RyRs. Expression of RyRs, as the major SR Ca^{2+} release channel in cardiac myocytes, is large, amounting to ~500–1000 fmol/mg total protein in ventricular myocytes from various mammalian species [52]. This converts to ~1.5–3.5 million RyRs in a typical ~30 pl ventricular myocyte [53]. Direct comparison of the expression of RyRs and IP_3Rs in ventricular myocytes revealed that RyRs outnumber IP_3Rs by a factor between 50:1 and 100:1 [54]. Microsomal preparations from hearts of various species, including human, exhibit a much larger density of RyRs than IP_3Rs. For example, RyR densities amounted to ~3–4 pmol/mg protein in human ventricle [55,56] and to ~5.5–7 pmol/mg protein in dog, mouse, rabbit and rat ventricle [56]. IP_3R densities, on the other hand, were ~0.09 pmol/mg protein in bovine ventricle [16], 0.66 pmol/mg protein in canine ventricle [32], 0.46 pmol/mg protein in ferret ventricle [57] and 0.35 pmol/mg protein in rat ventricle [58]. Thus, on average there appear to be ~5–80 times less IP_3Rs than RyRs in ventricular SR. These data indicate that cardiac IP_3Rs face a serious problem; they have to generate a detectable Ca^{2+} signal against a large background of RyR-induced Ca^{2+} release. How can so few IP_3Rs have an impact on cardiac Ca^{2+} signaling, which is clearly dominated by RyRs? Or, put another way, how can cardiac myocytes decipher and make use of a small hormone-evoked IP_3-induced Ca^{2+} signal amidst the large action potential-evoked RyR-induced Ca^{2+} transients?

RyRs require cytoplasmic Ca^{2+} increases for their activation, and are thereby tuned to the opening of voltage-activated Ca^{2+} channels during each action potential [59]. IP_3Rs are opened by a combination of IP_3 and Ca^{2+} binding [60,61]. It is generally understood that binding of IP_3 allows IP_3Rs to be activated by Ca^{2+} [23]. The need for synergistic binding of both IP_3 and Ca^{2+} explains why IP_3Rs can contribute to systolic Ca^{2+} transients—they can sit with IP_3 bound and wait for an activating Ca^{2+} signal to arise from voltage-activated channels or neighboring RyRs. However, IP_3 binding not only allows IP_3Rs to be activated by Ca^{2+}, it also increases their sensitivity to Ca^{2+}. So, with substantial increases of intracellular IP_3 concentrations as may occur following stimulation of cardiomyocytes with G_q protein-coupled agonists, IP_3Rs can become activated at normal diastolic Ca^{2+} levels, or during the recovery of a stimulated Ca^{2+} transient [62]. The promiscuous opening of IP_3Rs during the otherwise quiescent diastolic period under these conditions is therefore the cause of arrhythmic Ca^{2+} transients and their associated electrical activity [63,64].

5. General considerations on IP_3 and intracellular Ca^{2+} homeostasis

Since IP_3 releases Ca^{2+} from Ca^{2+} stores within cardiac myocytes, it affects cellular Ca^{2+} homeostasis. Cellular Ca^{2+} homeostasis is governed by influx and efflux of Ca^{2+} into and out of the cell, respectively, by intracellular Ca^{2+} buffering as well as by Ca^{2+} storage in, distribution between and release from intracellular compartments. The major Ca^{2+} store in cardiac myocytes is the SR. Depending on species and experimental conditions, SR Ca^{2+} release contributes ~50–90% of Ca^{2+} to the electrically stimulated (action potential-induced) global Ca^{2+} transient [53]. For sustained increases of Ca^{2+} transients to occur, SR Ca^{2+} load and hence the amount of Ca^{2+} released from the SR during an action potential have to increase. SR Ca^{2+} load is governed by the balance between Ca^{2+} release or "leak" from the SR and Ca^{2+} uptake into the SR. The former is mediated mainly by SR Ca^{2+} release channels, i.e. RyR2 and IP_3Rs, the latter by the SR Ca^{2+} pump (SERCA2a), which is regulated by phospholamban. Elegant quantitative studies have shown that it is not sufficient to enhance SR Ca^{2+} release alone to arrive at a sustained increase of the Ca^{2+} transient. Rather, uptake has to be increased in parallel (as occurs, for example, during β-adrenergic stimulation), because of SR Ca^{2+} autoregulation [65,66]. For example, when RyR2 open probability is increased selectively (through application of low concentrations of caffeine), global Ca^{2+} transients are enhanced at first, but then decline to reach steady-state amplitudes that are identical to the pre-caffeine amplitudes [67]. This is because some of the extra Ca^{2+} released from the SR is exported from the cell through sarcolemmal mechanisms (i.e. largely NCX), which reduces SR Ca^{2+} load. Reduced SR Ca^{2+} load, in turn, limits SR Ca^{2+} release because of the steep dependence of release on load [68], which is ultimately caused by the dependence of RyR2 open probability on SR luminal Ca^{2+} concentration [69]. A similar situation might arise when IP_3 concentration is elevated (e.g. by G_q protein-coupled agonists like endothelin-1). Under these conditions, the global Ca^{2+} transient is expected to increase because of the extra Ca^{2+} released from the SR by activated

IP_3Rs. Because of the larger Ca^{2+} transients, there will be a net loss of Ca^{2+} from the cell via sarcolemmal NCX. This should reduce SR Ca^{2+} load and hence limit the steady-state amplitude of the Ca^{2+} transient to the amplitude reached before elevation of IP_3 concentration took place. This is, however, not what is observed experimentally. As outlined below, exposure of cardiac myocytes to G_q protein-coupled agonists or membrane-permeable analogues of IP_3 causes a sustained increase in global Ca^{2+} transients, e.g. [63,70–72], with SR Ca^{2+} load staying unchanged or even tending to decrease [72]. Thus, IP_3 uniquely alters cardiac excitation–contraction coupling in that it increases Ca^{2+} transients and fractional SR Ca^{2+} release at largely unaltered SR Ca^{2+} load. This finding implies that, in addition to increasing SR Ca^{2+} release during systole through activation of IP_3Rs, IP_3 affects cellular Ca^{2+} homeostasis in yet another way. In fact, G_q protein-coupled agonists or IP_3 may also increase diastolic cytoplasmic Ca^{2+} concentration [17,70] and alter the kinetics of the cytoplasmic Ca^{2+} transient with a prolongation of the rising phase and an acceleration of the decay [72]. Clearly, future studies should aim at analyzing IP_3 effects on cellular Ca^{2+} fluxes in a more quantitative way, which will help gain more insight into the actions of IP_3 on cellular Ca^{2+} homeostasis.

6. Elementary IP_3-induced Ca^{2+} release events in cardiac myocytes

Discrete, non-propagating elementary Ca^{2+} release events have been characterized in a variety of excitable and non-excitable cell types (for reviews see [73,74]), for both RyRs ('Ca^{2+} sparks'; [75–78]) and IP_3Rs [70,79,80]. Ca^{2+} sparks represent Ca^{2+} release from a restricted number of clustered RyRs opening in concert [81]. It is well established that Ca^{2+} sparks represent the elementary Ca^{2+} release events that spatially and temporally summate to produce the global Ca^{2+} transient during cardiac excitation–contraction coupling.

IP_3R-dependent elementary Ca^{2+} release events, termed 'Ca^{2+} blips' (single channel event) and 'Ca^{2+} puffs' (multi-channel event originating from a group of IP_3Rs), have been observed in non-excitable cells such as oocytes [79], HeLa [82] and vascular endothelial cells [80]. Ca^{2+} puffs differ from RyR-mediated Ca^{2+} sparks in having significantly slower kinetics. Despite the growing evidence that IP_3R-evoked Ca^{2+} release occurs in cardiac cells, reports of elementary Ca^{2+} signals arising from IP_3Rs in cardiac myocytes have been scarce. This is likely related to the significantly lower density of IP_3Rs, which practically means that Ca^{2+} puffs are difficult to discern in the 'Ca^{2+} noise' from RyR-dominated Ca^{2+} release. A recent report showed that this problem could be circumvented experimentally by eliminating Ca^{2+} release via RyRs pharmacologically [70]. This is illustrated in Fig. 1. Permeabilized cat atrial myocytes displaying spontaneous Ca^{2+} release activity were treated with the RyR inhibitor tetracaine [83], to block Ca^{2+} sparks. The cells were then exposed to IP_3, which resulted in the appearance of localized non-propagating Ca^{2+} elevations that were completely abolished by the IP_3R blocker heparin. In comparison to typical Ca^{2+} sparks, the IP_3-evoked Ca^{2+} puffs had amplitudes which were 75–80% smaller and were about 3 times longer-lasting, and their rise time was prolonged approximately twofold. The spatial spread of the RyR- and IP_3R-mediated elementary events did not differ significantly (for average numbers see Fig. 1D). However, the underlying Ca^{2+} release flux was clearly smaller for Ca^{2+} puffs compared to Ca^{2+} sparks and the declining phase of $[Ca^{2+}]$ was prolonged (Fig. 1C). In summary, the IP_3-dependent elementary Ca^{2+} release events in permeabilized atrial myocytes were distinctly different from Ca^{2+} sparks and were reminiscent of Ca^{2+} puffs typically observed in non-excitable tissue where IP_3-dependent Ca^{2+} signaling is predominant [79,80,82,84]. These data are consistent with the notion that IP_3Rs provide a significantly smaller Ca^{2+} release pathway compared to RyRs. However, their relative paucity does not eliminate their potential to affect cardiac Ca^{2+} signaling. It is plausible that IP_3R-dependent Ca^{2+} puffs can summate with RyR-mediated Ca^{2+} sparks to increase the amplitudes of electrically-evoked Ca^{2+} transients. In addition, Ca^{2+} puffs may activate neighboring RyRs via Ca^{2+}-induced Ca^{2+} release and thereby amplify SR Ca^{2+} release. Alternatively, IP_3-mediated Ca^{2+} signals may have distinct functions of their own due to spatial separation from the machinery underlying excitation–contraction coupling. These possibilities are discussed further below.

7. IP_3 signaling in cardiac development

The heart is one of the first organs to develop in a growing embryo. As the heart is formed, its cells acquire the abilities to generate pulsatile Ca^{2+} signals, to become electrically active and to contract. A number of studies, using either pre-natal cardiac cells or embryonic stem cell-derived myocytes, have indicated that IP_3Rs may play a significant role in the development of the heart by contributing to the initiation of pacemaking activity, and thereby promote cardiogenesis. Spontaneous Ca^{2+} oscillations begin as soon as the heart tube is created. These Ca^{2+} signals are necessary to drive contraction, as well as appropriate gene transcription and structural arrangement of the nascent cardiomyocytes [85]. Indeed, inhibition of the Ca^{2+} oscillations or buffering the Ca^{2+} changes prevents development of the heart [86].

As described above, type 2 RyRs underlie the release of Ca^{2+} during systole in adult and neonatal cells. Mice lacking type 2 RyRs die as embryos around E10 with major cardiac defects [87] because the excitation–contraction coupling system cannot mature. However, repetitive Ca^{2+} signals and rhythmic contractions occur in these RyR-deficient animals at E7–9.5 [87,88]. In addition, the Ca^{2+} signals in young embryonic heart cells are insensitive to experimental maneuvers that inhibit voltage-activated Ca^{2+} channels or Na^+/Ca^{2+} exchange, but are affected by agents that deplete intracellular Ca^{2+} stores [89] (but see [90] for an alternative view). Taken together, these data indicate that periodic Ca^{2+} release from intracellular stores occurs in young embryonic heart cells, and that the channels involved are not RyRs.

There is accumulating evidence that IP_3Rs may be responsible for the early cycling of Ca^{2+} in developing myocytes prior to the maturation of excitation–contraction coupling. For example, the spontaneous Ca^{2+} oscillations in murine

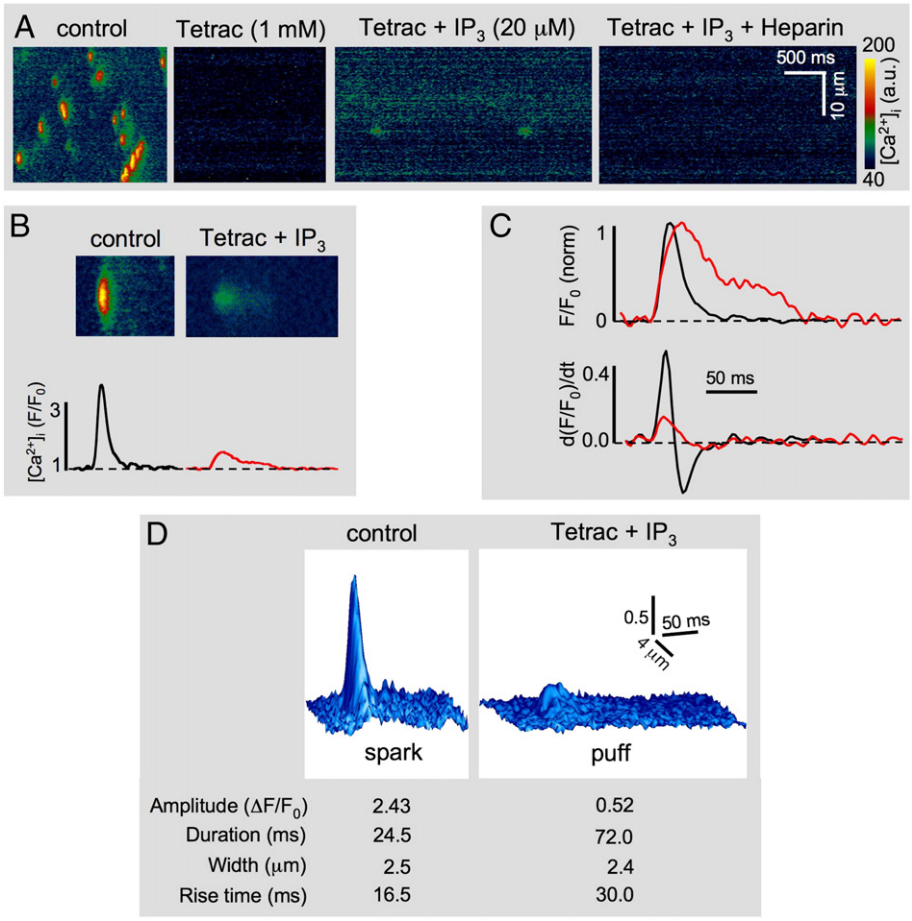

Fig. 1. Elementary IP_3-induced Ca^{2+} release events in cardiac myocytes. (A) Confocal linescan image of Ca^{2+} sparks in a permeabilized cat atrial myocyte (control). Tetracaine (Tetrac) abolishes Ca^{2+} sparks. Addition of IP_3 elevates baseline Ca^{2+} concentration and causes the appearance of smaller Ca^{2+} release events, i.e. Ca^{2+} puffs, which are suppressed by heparin. (B) Averaged Ca^{2+} spark and Ca^{2+} puff. (C) Normalized Ca^{2+} spark (black) and Ca^{2+} puff (red) (top) and first derivative thereof (bottom), a measure for the underlying Ca^{2+} release flux. The Ca^{2+} puff exhibits slower kinetics and lower Ca^{2+} release flux than the Ca^{2+} spark. (D) Surface plots of averaged linescan images of Ca^{2+} sparks and Ca^{2+} puffs. The Ca^{2+} puff displays lower amplitude, longer rise time and duration, and similar width as compared to the Ca^{2+} spark. Modified from [70]. Copyright of the Journal of Physiology; used by kind permission.

embryonic cells were sensitive to application of 2-APB (an IP_3R antagonist [42]) [89]. Furthermore, IP_3Rs are expressed in the inner mass of embryos at E5.5, and are clearly evident within the developing heart tube at E8.5 [91]. In contrast, RyRs are not abundant until E8.5 or later [91]. These data indicate that IP_3Rs are expressed earlier than RyRs in the developing heart, and the timing of their expression correlates closely with the on-set of Ca^{2+} oscillations.

Stem cell-derived myocytes grown *in vitro* can recapitulate the development and differentiation of cardiomyocytes very closely [92–94]. The accessibility of these cells and the relative ease by which they can be genetically manipulated (compared to myocytes in early embryos) has led them to be used for many studies of cardiac development. It is established that these cells express IP_3Rs when they are in either a proliferative state or during differentiation into cardiomyocytes [95]. Similar to their *in vivo* counterparts, stem cell-derived myocytes start to show spontaneous Ca^{2+} oscillations, membrane depolarizations and contractions long before the cells have terminally differentiated. The mechanism underlying the acquisition of spontaneous activity in these cells is not fully clear. Likely candidates could be the hyperpolarization-activated cyclic nucleotide-gated cation (HCN) channels that are responsible for pacemaking currents (I_f) in adult tissue [96]. However, the spontaneous Ca^{2+} oscillations appear to precede the appearance of substantial HCN-mediated currents, and the activity persists in the presence of I_f inhibitors [97]. Furthermore, repetitive Ca^{2+} transients could still be observed in cells that were fully depolarized (using K^+ application), thereby precluding the involvement of membrane potential oscillations [98]. In contrast, transfection of early stem cell-derived myocytes with antisense cDNA to reduce expression of type 1 IP_3Rs, or application of an IP_3R antagonist, significantly reduced the spontaneous beating [92,97]. The effect of antagonizing IP_3Rs diminishes over time [99], suggesting that IP_3-driven signals progressively give way to other mechanisms that generate Ca^{2+} oscillations [100]. As expected of a pacemaking messenger, stimulating IP_3 production in stem cell-derived myocytes using hormonal agonists had a positive chronotropic effect, which was antagonized by either IP_3R or phospholipase C inhibitors [101].

The studies described above suggest a fundamental role of IP_3Rs in triggering the first few days of Ca^{2+} cycling within the developing heart, and consequently allowing the cardiac cells to differentiate appropriately. After a short while, the Ca^{2+} cycling switches to a reliance on I_f, voltage-activated Ca^{2+} channels and RyRs. However, IP_3Rs are not entirely lost during fetal development. Indeed, neonatal animals have a relatively high expression of IP_3Rs (compared to adult cells), and the channels are demonstrably functional. For example, addition of IP_3 to permeabilized cells, or uncaging of photo-releasable IP_3, has directly demonstrated the presence of active IP_3Rs *in situ* within neonatal myocytes [102,103]. Furthermore, neonatal cardiac myocytes respond to several agonists (e.g. endothelin-1, angiotensin II, phenylephrine, ATP, prostaglandins, IGF-1) with IP_3 production and Ca^{2+} mobilization [102,104]. These same agonists also promote hypertrophic growth of neonatal cardiac myocytes [104,105]. Studies using immunostaining, Western blotting and analysis of mRNA have suggested that IP_3R types 1, 2 and 3 are expressed in neonatal cells [102,103,106].

A consistent message from the investigations of neonatal myocytes is that IP_3 causes perinuclear Ca^{2+} release. Almost all studies that examined either the distribution of IP_3Rs, or the spatial pattern of IP_3-evoked Ca^{2+} signals, have reported that the channels are largely expressed in close proximity to the nucleus in neonatal cells [102,103,106–108]. This is unlike RyRs, which have a more widespread expression pattern in neonatal cells [102]. The perinuclear release of IP_3-sensitive Ca^{2+} stores can impact specifically on nucleoplasmic Ca^{2+} levels [103] and thereby promote hypertrophic gene transcription [109].

The overall picture that emerges from the studies of embryonic tissues and stem cell-derived cardiomyocytes is that IP_3Rs are expressed in cardiac precursor cells, and are relatively abundant at the very earliest stages of heart development when they may play a critical role in development. Their expression declines as the cells differentiate and other Ca^{2+}-handling systems are put into place. However, IP_3Rs are not completely lost. They are retained in the heart for the duration of fetal development and can be readily demonstrated in neonatal cardiac myocytes, and their expression persists into adulthood.

It should be noted that mice deficient in cardiac expression of type 2 IP_3Rs are viable and do not have any apparent cardiac defects [17]. Furthermore, complete single deletion of type 1, type 2 or type 3 IP_3Rs in mice, or combined knockout of types 2 and 3 IP_3Rs, does not prevent the murine heart (or whole animal) from developing [110,111]. These data could indicate that IP_3Rs are not essential for cardiogenesis. However, it is possible that there is functional redundancy between IP_3R isoforms, such that the presence of any one of the three isoforms will do. Alternatively, another Ca^{2+} signaling mechanism could compensate for the normally undertaken by IP_3Rs. Although knockout of types 2 and 3 IP_3Rs appears to be benign, mice not expressing type 1 IP_3Rs mostly die *in utero* [110]. It would be interesting to know if a lack of cardiac development was partially responsible for the increased mortality of these animals.

8. IP_3 signaling in atrial myocytes

There is substantial evidence for IP_3-mediated Ca^{2+} signaling in atrial cells. The atrial myocardium expresses numerous receptors coupling to the PIP_2-PLC-IP_3 cascade. Increased phosphoinositide breakdown and inositol phosphate generation in atrium is mediated by receptors for acetylcholine (muscarinic, M_1 and M_3), angiotensin II (AT_1), endothelin (ET_A), histamine (H_1), 5-hydroxytryptamine (5-HT_2), norepinephrine (α_1) and vasopressin in various species including cat, chick, guinea-pig, mouse, rat and human [10,112–125]. Furthermore, expression of IP_3Rs in atrial myocytes has been demonstrated at mRNA and protein levels in various organisms, including mouse, rabbit, rat and human [15,17,41,58]. Myocytes from animal atrium are particularly rich in type 2 IP_3Rs and may also express type 3 IP_3Rs [41]. Human atrial myocytes, on the other hand, have been suggested to express predominantly type 1 IP_3R [15], although this requires further clarification.

Early evidence for an involvement of IP_3 in the regulation of atrial contraction came from the observation that phosphoinositide breakdown and inositol phosphate generation were accompanied by a positive inotropic effect [114–117,119–121]. Direct application of IP_3 to permeabilized atrial muscle [50], photorelease of IP_3 [126] and, more recently, exposure of isolated atrial myocytes to a membrane permeable analogue of IP_3 [58,63,127,128] provided definitive proof that IP_3 induces a positive inotropic effect in atrium. Thus, an important action of IP_3 in atrium is to increase the force of contraction.

Interestingly, receptor-mediated inositol phosphate production and expression of IP_3Rs are larger in atrium than in ventricle [10,17,41,58,113]. For example, maximal muscarinic receptor-mediated inositol phosphate accumulation was >12-fold in guinea-pig atrial myocytes but only 7-fold in ventricular myocytes [10]. Similarly, in cat myocardium, muscarinic receptor stimulation induced larger increases in phosphatidylinositol breakdown in atria than in ventricles [113]. In rat and rabbit myocytes, expression of IP_3Rs is ~3.5–10 times larger in atrial as compared to ventricular myocytes [41,58]. Microsomal preparations from human and sheep atrium displayed RyR densities of ~0.3–0.7 pmol/mg protein [129,130]. In contrast, IP_3R density amounted to 1.8 pmol/mg protein in rat atrium [58]. Thus, atrial SR contains more IP_3Rs, but less RyRs, than ventricular SR, suggesting that SR Ca^{2+} release in atrial myocytes is more dependent on IP_3 signaling than in ventricular myocytes. In line with this notion, direct application of IP_3 to permeabilized rat myocytes revealed a ~5 times larger global Ca^{2+} increase in atrial than in ventricular myocytes [58]. Taken together, these data might explain, at least in part, why many transmitters and hormones coupling to the PIP_2-PLC-IP_3 cascade exert comparatively large positive inotropic effects in the atrium but only small or no positive inotropic effects in the ventricle (e.g. angiotensin II [131] or 5-hydroxytryptamine [124]).

IP_3 does not alter myofilament Ca^{2+} responsiveness [48]. Rather, the positive inotropic effect of IP_3 in atrial myocardium is mediated by enhanced Ca^{2+} release from the SR. The extra component of Ca^{2+} mobilization from IP_3Rs adds to the action

potential-induced RyR-mediated Ca^{2+} release, and thereby increases the Ca^{2+} transient underlying contraction. Potentiation of Ca^{2+} transients following activation of IP_3Rs has been observed during stimulation of cat, mouse, rabbit and rat atrial myocytes with endothelin-1 or a membrane-permeant form of IP_3 (IP_3BM) [17,63,70–72,127,128]. The increase in the electrically-stimulated Ca^{2+} transient occurred without alteration of the Ca^{2+} load within the SR [72]. Thus, IP_3 increased fractional Ca^{2+} release from the SR (by ~13%) and did not affect SR Ca^{2+} storage *per se* [17,72].

IP_3-induced Ca^{2+} release may also lead to arrhythmogenic alterations in atrial Ca^{2+} signaling. In cat and rabbit atrial myocytes, endothelin-1 induced Ca^{2+} alternans [70,132], which may degenerate into arrhythmogenic Ca^{2+} waves [133]. Furthermore, endothelin-1 or IP_3BM elicited spontaneous Ca^{2+} release events during diastole in cat, mouse, rabbit and rat atrial myocytes. These spontaneous signals ranged from spark-like events and Ca^{2+} waves to extra action potential-derived global Ca^{2+} transients [63,70,71,128,132]. Similarly, in human atrial myocardium, endothelin-1 induced arrhythmic extra contractions mediated by activation of PLC and IP_3Rs [134,135] and in isolated human atrial myocytes angiotensin II, another agonist coupling to PLC–IP_3 signaling, increased the frequency of spontaneous Ca^{2+} sparks without altering SR Ca^{2+} load [136].

IP_3-induced arrhythmogenic Ca^{2+} release likely involves the recruitment of RyRs via Ca^{2+}-induced Ca^{2+} release. In rat atrial myocytes, some IP_3Rs and RyRs are colocalized in the subsarcolemmal area closely apposed to the sarcolemmal Na^+/Ca^{2+} exchanger (NCX), as illustrated in Fig. 2. The proximity of the two types of Ca^{2+} channels makes it likely that activation of IP_3Rs will provoke RyR opening. The consequent Ca^{2+} signal would then activate the nearby NCX, thereby generating a depolarizing current. Recruitment of multiple IP_3R/RyR clusters, during hormonal stimulation, can lead to sufficient NCX activation for a full depolarization of the cell. In turn, cellular depolarization will evoke a global diastolic Ca^{2+} transient. In support of this scheme, it has been demonstrated that the spontaneous diastolic action potential-evoked Ca^{2+} transients observed during endothelin-1 or IP_3BM application are preceded by subsarcolemmal Ca^{2+} release events (probably IP_3R-activated Ca^{2+} sparks; [63]). Furthermore, in human atrial myocardium, inhibition of RyRs suppressed endothelin-1-induced arrhythmias. These data suggest that the relatively few IP_3Rs in the atrium can cause a disproportionately large Ca^{2+} release due to amplification from nearby RyRs, and thereby induce NCX-triggered arrhythmias. Since reduction of SR Ca^{2+} load did not affect the arrhythmias [135] and, moreover, endothelin-1 did not alter the SR luminal Ca^{2+} content [17,72], SR Ca^{2+} overload can be ruled out as a causative factor [135]. Thus, atrial arrhythmias elicited by IP_3 appear to be distinctly different from classical triggered arrhythmias as may occur following digitalis exposure or excessive β-adrenergic stimulation. Although both are initiated by diastolic SR Ca^{2+} release and subsequent activation of NCX, in the former case Ca^{2+} release occurs at *normal* SR Ca^{2+} load whereas in the latter case Ca^{2+} release is the result of SR Ca^{2+} *overload*.

IP_3Rs are distributed not only in the subsarcolemmal/cytoplasmic compartment of atrial cells, but also in the perinuclear area, giving rise to the notion that they might control nuclear Ca^{2+}. Direct functional evidence for this has been obtained recently in cat, rabbit and rat atrial myocytes [71,72,137]. In permeabilized cat atrial myocytes (Fig. 3A), IP_3 or adenophostin elicited increases in nuclear Ca^{2+} that could be blocked by IP_3R antagonists [137]. In isolated nuclei (from whole hearts, Fig. 3B), this IP_3-induced Ca^{2+} release was observed both at the outer and inner face of the nuclei suggesting that functional IP_3Rs may be expressed at the outer and inner sides of the nuclear envelope facing the cytoplasm and the nucleoplasm, respectively [137]. Conversely, when the nuclear envelope was loaded with the low affinity Ca^{2+} dye fluo-5N to measure nuclear envelope Ca^{2+} concentration directly (Fig. 3C), IP_3 reduced the Ca^{2+} stored in the nuclear envelope [137]. Consistent with these findings from permeabilized myocytes and isolated nuclei, in electrically stimulated intact atrial myocytes, endothelin-1 augmented nuclear Ca^{2+} transients through IP_3 signaling beyond the increase seen in the cytoplasm [71,72]. At threshold concentrations, endothelin-1

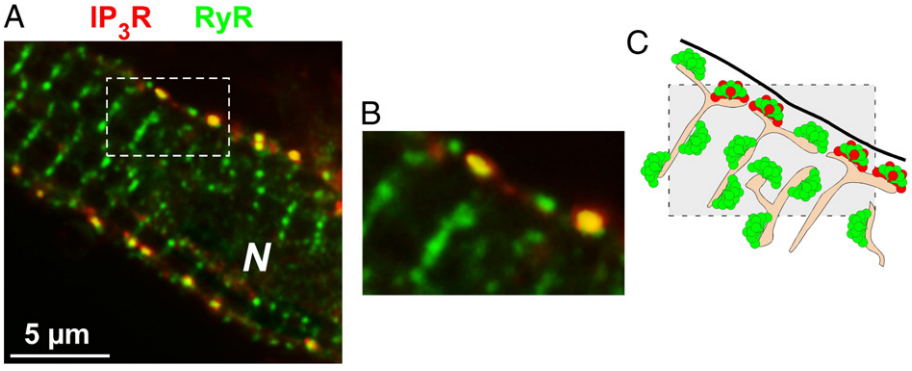

Fig. 2. Colocalization of subsarcolemmal IP_3Rs and RyRs in atrial myocytes. (A) Confocal image of a rat atrial myocyte immunostained for the expression of IP_3Rs (red) and RyRs (green). *N* denotes the nucleus. The area marked by the dashed white rectangular is shown enlarged in (B). Colocalization of IP_3Rs and RyRs (yellow) occurs in some areas underneath the sarcolemma. (C) Schematic illustration of colocalization of subsarcolemmal IP_3Rs and RyRs. Modified from [63]. Copyright of the Journal of Physiology; used by kind permission.

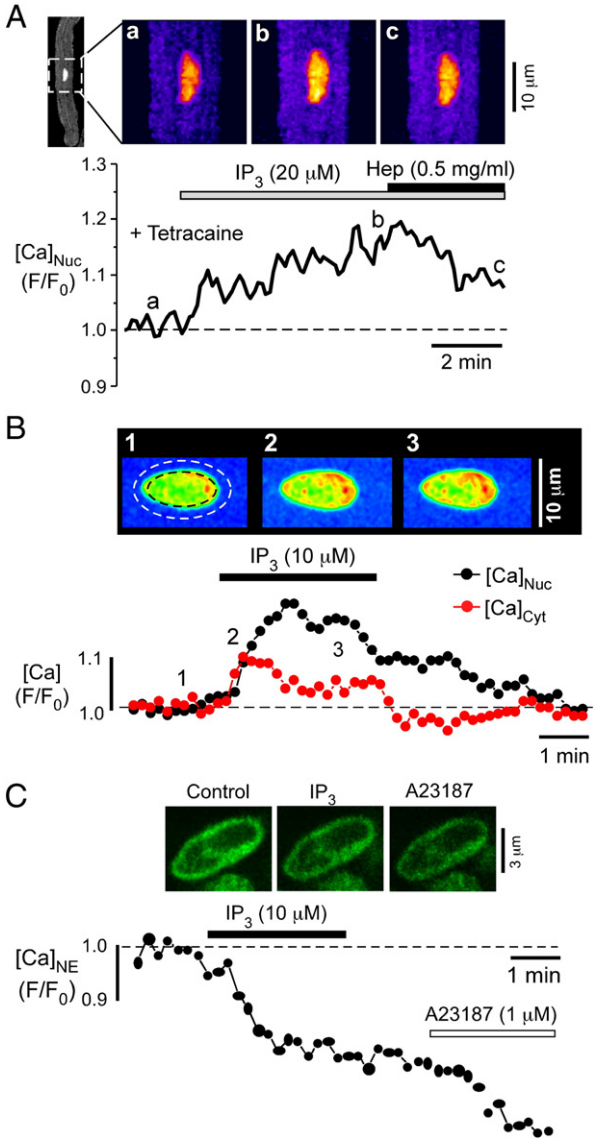

Fig. 3. IP_3-dependent nuclear Ca^{2+} signaling in cardiac myocytes. (A) Confocal images of a permeabilized cat atrial myocyte. In the presence of 0.7 mM tetracaine, IP_3 increases nuclear Ca^{2+} concentration. The IP_3-induced nuclear Ca^{2+} increase is partly reversed by the addition of heparin (Hep). (B) Confocal images of an isolated rat cardiac nucleus. The nucleus and the solution surrounding the nucleus contained fluo-4 dextran. Fluorescence changes from the regions marked by the dashed black line (nucleus) and the area delimited by the nuclear border and the dashed white line ('cytoplasm') are shown below. The IP_3-induced Ca^{2+} increase is larger in the nucleus (black) than in the adjacent cytoplasm (red). (C) Confocal images of an isolated rat cardiac nucleus. The nuclear envelope (NE) has been loaded with the low affinity Ca^{2+} dye fluo-5N. Addition of IP_3 causes a reduction of Ca^{2+} stored in the NE. The Ca^{2+} ionophore A23187 further depletes NE Ca^{2+}. Modified from [137]. Copyright of the Journal of Physiology; used by kind permission.

even induced a selective increase in nuclear Ca^{2+} transients [72].

Collectively, these data show that in atrium IP_3 may not only increase contractility via elevated Ca^{2+} transients, but that it also may induce arrhythmias and alter nuclear Ca^{2+} signaling to promote remodeling and regulate excitation–transcription coupling. Thus, it appears as if the adverse actions of atrial IP_3 signaling outweigh the beneficial effects. Clearly, further work is required to define more precisely the physiological and pathological roles of IP_3 signaling in atrial myocardium.

9. IP_3 signaling in ventricular myocytes

There is abundant evidence that IP_3Rs are expressed in mammalian ventricular myocytes [19,58,138]. IP_3R expression has been qualified using real-time PCR [58], Northern blotting [19,91], Western blotting [17,41,58], immunofluorescence [18,41,139] and IP_3 binding [58,139]. A number of studies have shown that ventricular myocyte IP_3Rs are functional and have access to replete Ca^{2+} stores *in situ*. For example, IP_3 has been shown to directly release Ca^{2+} from permeabilized/fractionated ventricular cells [41,140,141]. In addition, IP_3Rs have been purified from ventricular myocytes, incorporated into lipid membranes and shown to open after addition of IP_3 [57]. Although published data have suggested that all three IP_3R isoforms are expressed in ventricular myocytes, the majority of recent reports concur that type 2 IP_3Rs constitute the predominant channel isoform [17,18,41,58].

Despite the numerous lines of evidence for their expression and activity, the functional roles of ventricular IP_3Rs are still unclear. Although it is apparent that IP_3Rs can mobilize Ca^{2+} from stores within ventricular myocytes [41,140], it is not yet clear what and where these stores are. Furthermore, it is striking that IP_3Rs could have any significant effect on myocyte behavior in midst of the large Ca^{2+} fluxes that occur from RyRs during each heartbeat. Ventricular myocyte IP_3Rs are ~5 times less abundant than in atrial cells [17,41,58], and it is generally estimated that IP_3Rs are outnumbered by RyRs by a ratio of ~100:1.

The most obvious function for IP_3Rs would be to act as an inotropic support for the ventricles by boosting the amplitude of systolic Ca^{2+} transients, and thereby increasing contractile force. Consistent with this notion, IP_3Rs have been localized close to the dyadic junctions where excitation–contraction coupling is initiated [142], and direct activation of IP_3Rs was found to enhance Ca^{2+} spark occurrence throughout ventricular cells [41]. Furthermore, application of hormones that activate the production of IP_3 inside ventricular myocytes, such as endothelin-1 [11], enhances systolic Ca^{2+} signals in rat and rabbit cells [41,128,143]. IP_3 can therefore be considered as a *bona fide* inotropic agent in some mammalian ventricular myocytes (but perhaps not all; see [41]). However, while positive inotropy is a beneficial effect of IP_3R activation, the situation is not that straightforward. Rather than reinforcing their positive contribution to inotropy, the overwhelming majority of studies that have examined the consequence of activating IP_3Rs in ventricular myocytes have concluded that the predominant effect is to stimulate arrhythmias [62,143]. Indeed, IP_3R activity has been suggested to underlie ventricular arrhythmias resulting from hormonal stimulation [143], reperfusion [144,145], engagement of cytotoxic T-lymphocytes [146] and FAS receptor activation [147]. These data suggest that Ca^{2+} flux through the relatively few IP_3Rs in ventricular myocytes is perhaps more dangerous than beneficial, similar to the situation discussed above for atrial cells.

An intriguing clue to another possibly significant physiological/pathological function of IP_3Rs in ventricular myocytes is their localization close to the nucleus. A substantial proportion of IP_3Rs in adult ventricular cells is expressed on unspecified membranes close to the nucleus, or indeed on the nuclear envelope [18,148]. Furthermore, it has been shown that IP_3 can release Ca^{2+} from the ventricular myocyte nuclear envelope (and also probably from adjacent connected membrane compartments) [140,141]. This strategic positioning of a population of nucleus-associated IP_3Rs potentially allows the generation of autonomous nuclear Ca^{2+} signals, which may have a significant role in regulating cardiac gene transcription, and plausibly controlling processes such as cardiac hypertrophy. Indeed, it has been demonstrated that the release of perinuclear IP_3-sensitive Ca^{2+} stores promotes a CaMKII-dependent phosphorylation of histone deacetylase 5 (HDAC5) thereby causing it to be exported out of the nucleus [140]. Thus, perinuclear IP_3-evoked Ca^{2+} signals can derepress the expression of genes that underlie hypertrophic growth, such as those under the control of MEF2c [149]. It is well known that stimulation of myocytes with hormones that activate IP_3 production, e.g. ET-1, can promote hypertrophy [150–154]. What is not fully understood, however, is how such hormones can affect subtle changes in Ca^{2+}-dependent gene transcription inside cells that experience periodic surges of Ca^{2+} during excitation–contraction coupling [155]. Perinuclear IP_3Rs may provide a resolution to this conundrum by providing a source of Ca^{2+} signals that can be spatially and temporally dissociated from those that regulate contraction [156]. Consistent with this model, it has been demonstrated that activation of IP_3Rs in rat neonatal ventricular myocytes using a membrane-permeant IP_3 analogue or an α_1-adrenergic agonist causes perinuclear Ca^{2+} release events, and that blocking IP_3R opening decreases hypertrophic growth in response to phenylephrine stimulation [109]. Similar findings were made using mouse neonatal cells stimulated with ET-1 and phenylephrine; nuclear-associated IP_3Rs triggered nucleoplasmic Ca^{2+} signals and hypertrophic gene transcription [157]. Further work is required to establish whether this scheme is relevant *in vivo*. However, the concept that IP_3Rs impact specifically on nuclear Ca^{2+} signaling is gaining momentum. Numerous recent reports examining Ca^{2+} signaling in neonatal [103,108,109,157], atrial [71,72,137] and ventricular myocytes [140] have demonstrated specific effects of IP_3-generating agonists or IP_3 itself on nucleoplasmic Ca^{2+} signals. Coupling these data with the observations that IP_3 is necessary for some forms of hypertrophy [109,157,158] raises the possibility that IP_3Rs are key regulators of cardiac myocyte gene transcription and fate.

10. IP_3Rs in other cardiac cells

The majority of studies examining cardiac IP_3R expression and function have focused on developing embryonic/neonatal cells, or adult atrial and ventricular myocytes. However, there are reports suggesting that IP_3Rs are also expressed in other cardiac regions/cell types, such as papillary muscle [48,159]. It has also been proposed that IP_3Rs are functional in nodal cells within the developing atria. Isolation of atrial cells from E14.5 mouse embryos revealed a minor population of spontaneously active myocytes (presumed to be pacemaking cells). Transfection of an enzyme that metabolizes IP_3, and thereby prevents its Ca^{2+}-mobilising action, or an anti-IP_3R antibody, inhibited the spontaneous activity [97]. In the sinus node of the adult mammalian heart, spontaneously active pacemaker cells make use of cyclic subsarcolemmal RyR-mediated SR Ca^{2+} release to activate depolarizing NCX current, which contributes to diastolic depolarization and pacemaker activity (for review see [160]). This 'intracellular Ca^{2+} clock' is fueled by high, cAMP-dependent Ca^{2+} turnover. Increases and decreases, respectively, in intracellular cAMP levels modulate cycle length, i.e. heart rate, in part by alterations in PKA-dependent phosphorylation of phospholamban (and hence SR Ca^{2+} load and release). Because of this Ca^{2+} dependence of pacemaker activity, it appears feasible that IP_3R-mediated SR Ca^{2+} release might also modulate pacemaker activity in myocytes from adult sinus node. Direct evidence for an involvement of IP_3 signaling in the regulation of heart rate, however, is lacking. Angiotensin II and endothelin-1 (depending on species and experimental conditions), which are known to increase IP_3 in supraventricular tissue, exert positive chronotropic effects in mammalian heart via activation of AT_1 and ET_B receptors, respectively (e.g. [161,162]). Whether PLC–IP_3 signaling is involved in the positive chronotropic effects of angiotensin II and endothelin-1 is not known at present. Evidence from amphibian sinus venosus, however, suggests that PLC–IP_3 signaling and IP_3R-mediated SR Ca^{2+} release depolarize the membrane potential of pacemaker cells thereby increasing heart rate [163].

Purkinje cells also express IP_3Rs [164], seemingly to a greater extent than either atrial or ventricular myocytes [16]. The physiological function of IP_3Rs in these cells is unclear, but they may plausibly underlie the increased automaticity of Purkinje fibers during adrenergic stimulation [165] or following infarct [166]. Purkinje cells from infarcted hearts show proarrhythmic propagating Ca^{2+} waves that are reduced in frequency and amplitude by 2-APB. Also consistent with the notion of IP_3Rs regulating automaticity of Purkinje fibers, reduction of IP_3 production using a phospholipase C inhibitor was demonstrated to inhibit their autonomous Ca^{2+} spiking [102].

An overview on the expression of IP_3R isoforms in various regions of adult myocardium and the involvement of IP_3 signaling in excitation–contraction coupling, excitation–transcription coupling and arrhythmogenesis is provided in Table 1 and Fig. 4, respectively. Table 1 also lists evidence in favor of altered IP_3 expression in various cardiac diseases (for a more detailed discussion on this issue see below). It should be noted that, unlike RyR2, IP_3Rs are also expressed to a significant amount in non-myocytes in cardiac tissue (e.g. fibroblasts, endothelial and smooth muscle cells). Therefore, ideally expression of IP_3Rs and alterations thereof in cardiac disease should be confirmed in cardiac myocytes. Otherwise, the possibility exists that the findings are obscured by IP_3R

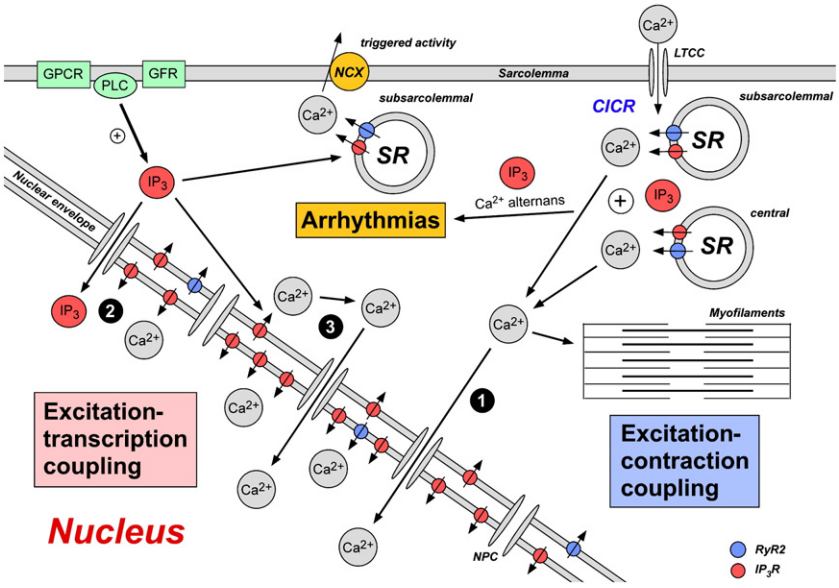

Fig. 4. Overview on IP_3 signaling and its involvement in excitation–contraction coupling, excitation–transcription coupling and arrhythmias in adult cardiac myocytes. Schematic drawing of an adult cardiac myocyte showing parts of the sarcolemma, the nucleus, the SR and the myofilaments. G protein-coupled receptors (GPCR), e.g. for angiotensin II, endothelin-1 or phenylephrine, as well as growth factor receptors (GFR) increase cytoplasmic IP_3 concentration via activation of phospholipase C (PLC). IP_3Rs are found in the SR (in atrial myocytes both in the subsarcolemmal and central SR) and in the nuclear envelope, where they face both the cytoplasm and the nucleoplasm. By contrast, RyR2s are found predominantly in the SR. IP_3 signaling is involved in excitation–contraction coupling, in excitation–transcription coupling and in the generation of arrhythmias. Excitation–contraction coupling is mediated by Ca^{2+}-induced Ca^{2+} release (CICR) from the SR through RyR2. Cytoplasmic Ca^{2+} binds to the myofilaments and initiates contraction. IP_3-induced Ca^{2+} release from the SR through IP_3Rs may contribute to excitation–contraction coupling by increasing the Ca^{2+} transient and thus contraction. On the other hand, IP_3-induced Ca^{2+} release from (subsarcolemmal) SR may trigger further Ca^{2+} release through neighboring RyR2 with subsequent activation of sarcolemmal NCX. This induces arrhythmias via generation of delayed afterdepolarizations and triggered activity. IP_3-induced SR Ca^{2+} release also generates pro-arrhythmic Ca^{2+} alternans. Finally, IP_3 is involved in excitation–transcription coupling via modulation of nuclear Ca^{2+} concentration. Nuclear Ca^{2+} may be increased by IP_3 in three ways: (1) IP_3 may increase the cytoplasmic Ca^{2+} transient. Cytoplasmic Ca^{2+} then diffuses through the nuclear pores into the nucleoplasm to elicit a delayed nuclear Ca^{2+} transient. (2) Cytoplasmic IP_3 may itself diffuse through the nuclear pores into the nucleus to activate nuclear IP_3Rs facing the nucleoplasm. Ca^{2+} release from the nuclear envelope, a Ca^{2+} store continuous with the SR, into the nucleoplasm then increases the nuclear Ca^{2+} concentration directly. (3) Finally, IP_3 may also activate IP_3Rs on the nuclear envelope facing the cytoplasm. Ca^{2+} release from the nuclear envelope into the cytoplasm may then increase nuclear Ca^{2+} concentration indirectly via cytoplasmic Ca^{2+} diffusing into the nucleus through nuclear pores. A fourth alternative (not shown in the cartoon) is the generation of IP_3 in the nucleus via nuclear GPCR coupling to the nuclear PLC-IP_3 cascade. Abbreviations: CICR, Ca^{2+}-induced Ca^{2+} release; GFR, growth factor receptor; GPCR, G protein-coupled receptor; LTCC, L-type Ca^{2+} channel; NPC, nuclear pore complex; NCX, Na^+/Ca^{2+} exchanger; PLC, phospholipase C.

expression from non-myocytes. For example, rat heart contains all three IP_3R isoforms with the IP_3R type 1 being the most abundant isoform (more than 60% of total IP_3R mRNA). Ventricular myocytes, however, do not contain IP_3R type 1 (less than 2% of total IP_3R mRNA), but rather express the type 2 (85%) and 3 (14%) IP_3R [57]. Thus, we have also indicated in Table 1 whether or not IP_3R expression was confirmed in cardiac myocytes.

11. Is nuclear Ca^{2+} regulated independently from cytoplasmic Ca^{2+}? Putative relevance of IP_3-dependent nuclear Ca^{2+} signaling for excitation–transcription coupling

As outlined above, there is clear evidence for regulation of nuclear Ca^{2+} concentration by IP_3 signaling. The question arises, however, whether nuclear Ca^{2+} in beating cardiomyocytes can be regulated independently from cytoplasmic Ca^{2+}, which undergoes large changes (from ~100 nM in diastole to >1 μM in systole) during each heartbeat. The nuclear pores, which traverse the nuclear envelope, represent a direct connection between cytoplasm and nucleoplasm through which Ca^{2+} can permeate. Consequently, increases in cytoplasmic Ca^{2+} during systole also result in increases in nuclear Ca^{2+} (see pathway #1 in Fig. 4). Due to a diffusional delay, the nuclear Ca^{2+} transient lags behind the cytoplasmic Ca^{2+} transient, as visualized by confocal Ca^{2+} imaging [71,72,167]. Hormone-independent increases in cytoplasmic Ca^{2+} transients (elicited by elevation of extracellular Ca^{2+}) are followed by increases in nuclear Ca^{2+} transients of similar magnitude [72]. Thus, cytoplasmic Ca^{2+} is an important determinant of nuclear Ca^{2+} arguing that nuclear Ca^{2+} in cardiac myocytes cannot be regulated entirely independently from cytoplasmic Ca^{2+}. IP_3 signaling, however, is able to *alter* nuclear Ca^{2+} *selectively* (see pathway #2 in Fig. 4). IP_3 can release Ca^{2+} from the nuclear envelope directly into the nucleoplasm through activation of IP_3Rs located at the inner face of the nuclear envelope [137]. This IP_3-induced Ca^{2+} release into the nucleus may be very local [140]. It can cause selective increases in the nuclear Ca^{2+} transient [72], as illustrated in Fig. 5, and activate nuclear CaMKIIδ_B to promote phosphorylation of HDACs and, thereby, transcription [140]. In this way, IP_3 can regulate nuclear Ca^{2+}-dependent processes like transcription independently from

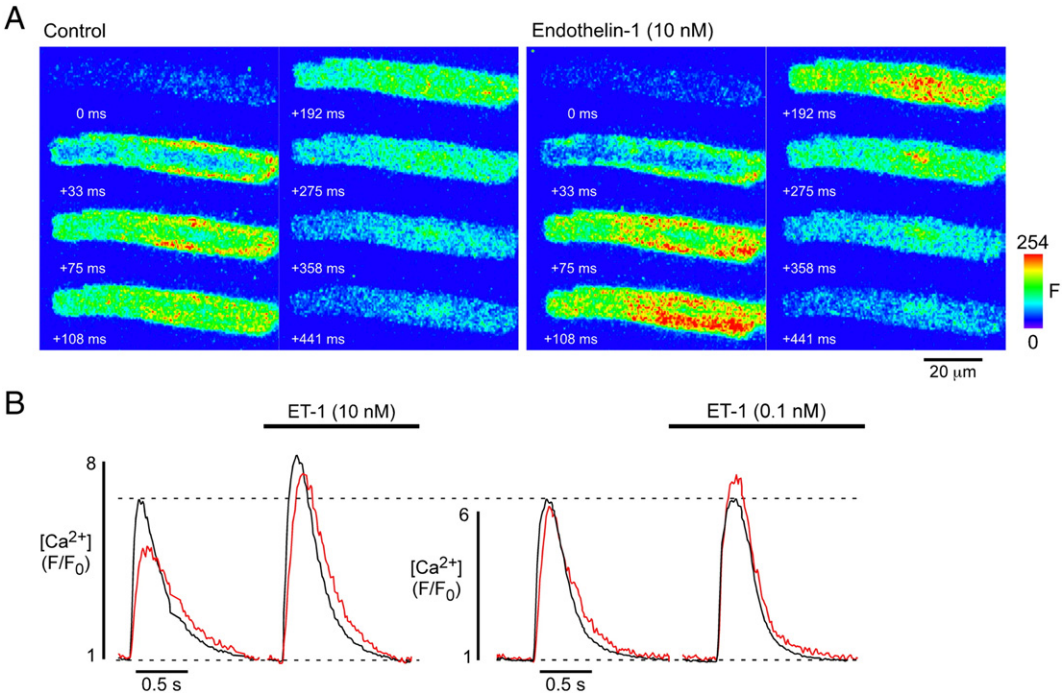

Fig. 5. Endothelin-1 enhances nuclear Ca^{2+} transients in atrial myocytes. (A) Two-dimensional confocal images of a rabbit atrial myocyte during an electrically-stimulated Ca^{2+} transient before (Control) and after the addition of 10 nM endothelin-1. The nucleus is visible as the oval area in the cell center with a delayed Ca^{2+} increase during the rising phase and with a delayed Ca^{2+} decrease during the decaying phase of the Ca^{2+} transient. (B) Ca^{2+} transients (from the cell shown in A) obtained from the entire cytoplasm (black) and the nucleus (red). Addition of 10 nM endothelin-1 (ET-1) increases the cytoplasmic and, even more pronounced, the nuclear Ca^{2+} transient. When a threshold concentration of 0.1 nM endothelin-1 is applied (right; different cell), the cytoplasmic Ca^{2+} transient remains unaltered, whereas the nuclear Ca^{2+} transient is augmented selectively. Modified from [72]. Copyright of the Journal of Cell Science; used by kind permission.d

cytoplasmic Ca^{2+}-dependent processes by selective elevation of nuclear Ca^{2+} concentration.

12. Pathological relevance of IP_3 signaling

IP_3-induced Ca^{2+} release from the SR and the nuclear envelope may initiate arrhythmias and alter gene expression to induce hypertrophy and heart failure, as discussed above. In line with this notion, many signaling systems, most notably the sympathetic, the renin–angiotensin and the endothelin system, are activated during cardiac diseases associated with arrhythmias, hypertrophy and heart failure along with alterations in the expression of cardiac IP_3Rs. Substantive evidence suggests that IP_3 signaling may be involved in the development and/or maintenance of atrial fibrillation, reperfusion arrhythmias, ankyrin-B-related arrhythmias, diabetic cardiomyopathy, hypertrophy and heart failure.

12.1. Atrial fibrillation

Atrial fibrillation (AF) is the most common arrhythmia. Its incidence increases with age, as does the expression of the IP_3R in the heart [168]. Expression of the IP_3R is augmented in a dog model of AF and in human AF [15,169,170]. Furthermore, cardiac tissue levels of endothelin and angiotensin II are elevated in cardiac diseases associated with AF. Both endothelin and angiotensin II increase IP_3 generation [122,123] and elicit arrhythmias in isolated human atrial myocardium [134,135]. Thus, endothelin- and angiotensin II-induced IP_3 signaling may be involved in the initiation and progression of AF. While endothelin receptor antagonists have not yet been tested for their therapeutic potential against AF, several recent clinical trials suggest that ACE inhibition and AT_1 receptor blockade reduce the incidence of AF in patients at risk of developing the arrhythmia (reviewed in [171]). Furthermore, mutations in the adaptor protein ankyrin-B, which is required for post-translational stability and SR targeting of IP_3Rs, are associated with AF (see below).

12.2. Reperfusion arrhythmias

Ischemia and reperfusion can cause arrhythmias and sudden cardiac death. Upon reperfusion after an ischemic insult, there is a surge of IP_3 concentration in cardiac myocytes. The transient increase in IP_3 concentration is the result of α_1-adrenergic receptor stimulation by norepinephrine, which is released from sympathetic nerve endings within the heart. The increase in IP_3 concentration correlates with the induction of arrhythmias, ventricular tachycardia and ventricular fibrillation [145]. Substances able to inhibit the IP_3 increase prevent the arrhythmias [145,172,173]. Inhibition of the Na^+/H^+ exchanger (NHE) and NCX is also effective against the development of the arrhythmias [174,175]. This suggests that Na^+ influx through NHE, followed by Na^+ exit and Ca^{2+} influx through reverse mode NCX leads to an increase in SR Ca^{2+} load. IP_3-induced SR Ca^{2+} release with subsequent activation of forward mode

NCX may then trigger the arrhythmias. Reperfusion-induced increase in IP_3 concentration has been observed in atria and ventricles from both rat and human hearts following stimulation with either norepinephrine or thrombin (e.g. [4,173,176,177]).

12.3. Arrhythmias related to ankyrin-B mutations

Ankyrin-B is a 220 kDa adaptor protein that directly interacts with the IP_3R in cardiac myocytes. High affinity interaction between ankyrin-B and IP_3R is required for post-translational stability and targeting of the IP_3R to SR microdomains closely opposed to neighboring T tubules [178,179]. Loss-of-function mutations in ankyrin-B cause reduction of IP_3Rs in specialized SR–T tubule junctions (distinct from the classical dyads) in cardiac myocytes and are associated with arrhythmias ranging from bradycardia, sinus arrhythmia, atrial fibrillation and catecholaminergic polymorphic ventricular tachycardia to ventricular fibrillation both in humans and in mice heterozygous for a null mutation in ankyrin-B (ankyrin-$B^{+/-}$ mice) [142,180]. In patients affected by ankyrin-B mutations, emotional or physical stress may lead to syncopes and sudden death. The cellular mechanisms underlying the ankyrin-B-related arrhythmias may involve altered Na^+ and Ca^{2+} homeostasis. The macromolecular complex in the SR–T tubule junctions is composed of ankyrin-B, IP_3R and the Na^+-dependent transporters NCX and Na^+/K^+ pump. Loss-of-function mutations in ankyrin-B disrupt this macromolecular complex. Cardiac myocytes from ankyrin-$B^{+/-}$ mice exhibit normal resting $[Ca^{2+}]$ levels and unaltered L-type Ca^{2+} currents but increased Ca^{2+} transients and an increased propensity toward catecholamine-induced arrhythmogenic SR Ca^{2+} release [142] pointing to SR Ca^{2+} overload as a causative factor. It has been suggested that IP_3Rs act as "Ca^{2+} pressure valves" releasing Ca^{2+} from the SR, which is subsequently eliminated by the nearby NCX [179]. Thus, ankyrin-B-related arrhythmias represent another example for the importance of the subcellular distribution of the IP_3R for cardiac function.

An interesting feature of the above hypothesis is that loss-of-function of IP_3Rs (or mis-targeting) appears to be responsible for the ankyrin-B-related arrhythmias, which may ultimately be triggered by RyR-mediated SR Ca^{2+} release due to SR Ca^{2+} overload. This is in striking contrast to the arrhythmias triggered directly by activation of IP_3Rs and IP_3R-induced SR Ca^{2+} release at normal SR Ca^{2+} load. Thus, both activation and loss-of-function of IP_3Rs may be involved in the generation of cardiac arrhythmias.

12.4. Diabetic cardiomyopathy

Diabetic cardiomyopathy is now recognized as a distinct clinical entity with characteristic structural and functional changes of the heart. Ca^{2+} homeostasis is altered in myocytes from diabetic hearts such that electrically stimulated Ca^{2+} transients are reduced and prolonged. These alterations are the result of changes in the expression and function of SR Ca^{2+} handling proteins; SERCA2a, NCX and RyR expressions are reduced, whereas phospholamban expression is increased and its phosphorylation decreased. As a consequence, SR Ca^{2+} load is reduced, and SR Ca^{2+} release and re-uptake are impaired [181,182]. Interestingly, an increased propensity toward arrhythmogenic afterdepolarizations has been observed in diabetic cardiomyocytes [182]. Insulin increases IP_3 concentration and triggers arrhythmic Ca^{2+} release events in myocytes from diabetic *ob/ob* mice – but not from wild-type mice – and this effect is mediated via IP_3 signaling [183]. IP_3R expression (type 1 and type 2 IP_3Rs) is unaltered in ventricles from *ob/ob* mice [183], but decreased in a diabetes model in the rat and in atrium from diabetic patients [184,185]. Both increased and decreased IP_3 production in response to α-adrenergic stimulation has been reported in diabetes [186,187]. PIP_2 levels and the expression and activities of PLC isoenzymes are reduced [188]. Taken together, these data indicate that altered receptor-PLC coupling, IP_3 generation and IP_3-induced SR Ca^{2+} release may contribute to impaired Ca^{2+} handling and arrhythmogenesis in diabetic cardiomyopathy.

12.5. Hypertrophy and heart failure

Many agonists of G_q protein-coupled receptors induce cardiac hypertrophy that eventually may deteriorate into heart failure. The best studied and, possibly, clinically most relevant examples include angiotensin II, endothelin-1 and norepinephrine acting predominantly via AT_1, ET_A and α_1-adrenergic receptors, respectively. Plasma and tissue levels of angiotensin II, endothelin-1 and norepinephrine and receptor densities are often increased in hypertrophy. However, a direct role for IP_3 signaling in cardiac hypertrophy has remained elusive until most recently, since the respective receptors couple to various signaling pathways each of which may culminate into hypertrophy.

Early evidence suggested that IP_3 signaling in hypertrophy may be unaltered or even impaired [189,190]. More recent studies, however, collectively point toward an important role of PLC-IP_3 signaling in the development and progression of hypertrophy. For example, in pressure overload-induced hypertrophy, PLC activity was elevated and norepinephrine-induced generation of IP_3 was increased [191]. Moreover, IP_3-induced Ca^{2+} release from the SR was larger in myocytes from hypertensive rats than from control rats [192]. In volume overload-induced hypertrophy, sarcolemmal PIP_2 levels were decreased, whereas PLCβ1 and PLCγ1 expression and activities and IP_3 levels were increased [193]. Furthermore, in hypertrophy induced by transient overexpression of a constitutively active $G_{q\alpha}$, PLCβ1 and PLCβ3 expressions were elevated [194]. Thus, PLC–IP_3 signaling and IP_3-induced Ca^{2+} release are enhanced in cardiac hypertrophy. These findings also provide an explanation for the progressive nature of cardiac hypertrophy: initiation of increased gene expression through nuclear IP_3-induced Ca^{2+} release appears to contribute to increased expression of the very machinery responsible for the generation of IP_3, thereby creating a positive feedback loop [195].

Heart failure is accompanied by neurohormonal activation, including the sympathetic nervous system, the renin–angiotensin system and the endothelin system. Cardiac tissue levels of

norepinephrine, angiotensin II and endothelin are elevated. Expression of the respective receptors (which couple to IP_3 formation) is altered. In human heart failure, expression of α_1-adrenergic receptors is unchanged (but their relative expression is increased due to a large downregulation of the predominating β_1-adrenergic receptors) [196], AT_1 receptors are downregulated (presumably due to increased angiotensin II levels [197]) and ET_A receptors are upregulated [198,199]. In animal models of heart failure, changes in phosphoinositide metabolism occur, including alterations in the abundance, expression and/or activity of PIP_2, PI-4 kinase, PIP-5 kinase, PLC isoforms and inositol phosphates [200,201]. Expression of IP_3Rs is increased in both human and animal heart failure, whereas RyR levels are reduced, indicating a shift toward more IP_3R-mediated Ca^{2+} release in the failing heart [19,202]. These data indicate that, in heart failure, there are complex changes in the transmitter and receptor systems coupling to IP_3 formation as well as in the regulation of IP_3Rs suggesting that IP_3-induced Ca^{2+} release is involved in the development and/or progression of the disease.

13. Unresolved questions and future perspectives

Recent years have seen tremendous advances in our understanding of IP_3 signaling in cardiac myocytes. There is no longer any doubt that IP_3 plays important physiological and pathological roles in the heart. These roles, however, are incompletely understood and remain to be defined more precisely. A puzzling aspect of IP_3 signaling in adult cardiac myocytes is that apparently it exerts more adverse than beneficial effects. Is IP_3 signaling in adult myocardium a mere remnant of the developing heart or does it serve unique functions beyond arrhythmogenesis and induction of (maladaptive) remodeling? Further important but still largely unresolved questions are: Why do cardiac myocytes express different IP_3R isoforms? How is expression of IP_3Rs regulated? What is the composition, subcellular distribution and functional role of IP_3R macromolecular complexes in cardiac myocytes? Which kinases and phosphatases regulate cardiac IP_3R phosphorylation and what is the functional impact of this regulation? By what other means is IP_3R function regulated? How exactly is IP_3 signaling involved in the development and progression of cardiac diseases? Future studies should aim at answering these questions. Clearly, IP_3 signaling in cardiac myocytes will continue being an important topic in cardiac physiology as well as cardiology. It certainly holds the promise for further surprises and excitement.

Acknowledgments

The authors' work was supported by grants from the Deutsche Forschungsgemeinschaft (DFG–PI414/1 and PI414/2, KFO 155, to BP & JK), the National Institutes of Health (NIH–HL80101 and HL62231 to LAB), the American Heart Association (AHA–AHA0530309Z to AVZ) and the British Heart Foundation and the BBSRC (to MDB and HLR). HLR is a Royal Society University Research Fellow.

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