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

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Abstract

Inositol 1,4,5-trisphosphate (IP₃) 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 IP₃ from phosphatidylinositol 4,5-bisphosphate (PIP₂). The major action of IP₃, which is hydrophilic and thus translocates from the membrane into the cytoplasm, is to induce Ca²⁺ release from endogenous stores through IP₃ receptors (IP₃Rs). Cardiac excitation–contraction coupling relies largely on ryanodine receptor (RyR)-induced Ca²⁺ release from the sarcoplasmic reticulum. Myocytes express a significantly larger number of RyRs compared to IP₃Rs (~100:1), and furthermore they experience substantial fluxes of Ca²⁺ with each heartbeat. Therefore, the role of IP₃ and IP₃-mediated Ca²⁺ signaling in cardiac myocytes has long been enigmatic. Recent evidence, however, indicates that despite their paucity cardiac IP₃Rs may play crucial roles in regulating diverse cardiac functions. Strategic localization of IP₃Rs in cytoplasmic compartments and the nucleus enables them to participate in subsarcolemmal, bulk cytoplasmic and nuclear Ca²⁺ signaling in embryonic stem cell-derived and neonatal cardiomyocytes, and in adult cardiac myocytes from the atria and ventricles. Intriguingly, expression of both IP₃Rs and membrane receptors that couple to PLC/IP₃ signaling is altered in cardiac disease such as atrial fibrillation or heart failure, suggesting the involvement of IP₃ signaling in the pathology of these diseases. Thus, IP₃ 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

1. The discovery of IP₃

A quarter of a century ago it was shown that *D-myo* inositol 1,4,5-trisphosphate (IP₃) releases Ca²⁺ from a non-mitochondrial internal Ca²⁺ store [1]. Since this hallmark discovery, IP₃ has emerged as a ubiquitous intracellular messenger, releasing Ca²⁺ from stores through activation of IP₃ receptors (IP₃Rs) in almost all eukaryotic cells. The major IP₃-sensitive intracellular Ca²⁺ store is the endoplasmic reticulum. However, IP₃ has also been shown to release Ca²⁺ stored in other compartments, such as the Golgi and the nuclear envelope [2]. In addition, IP₃Rs are present on the plasma membrane of some cell types, where they can gate Ca²⁺ influx [3]. A crucial role for IP₃-dependent Ca²⁺ 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, IP₃-induced Ca²⁺ release from the sarcoplasmic reticulum (SR) plays a key role in pharmacomechanical 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 IP₃ has been enigmatic. However, a number of recent reports have begun to unravel the physiological and potentially pathological actions of IP₃ within the heart.

2. The ABC of IP₃: where does it come from and where does it go?

IP₃ is generated by hydrolysis of phosphatidylinositol 4,5-bisphosphate (PIP₂) through phosphoinositide-specific phospholipase C (PLC). PIP₂ 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]. PIP₂ 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 (PIP₃), 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), PIP₂ and Ca²⁺ (PLCδ) or Ras (PLCε). Consequently, many transmitters, neurohormonal factors, hormones and other stimuli (e.g. stretch) may increase IP₃ concentration and activate IP₃-induced Ca²⁺ release in cardiac myocytes downstream of PLC activation. Upon stimulation, intracellular IP₃, 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 IP₃ increase, which is likely to be quite modest. Recently, the concentration of free IP₃ was estimated directly using a novel FRET-based biosensor. Following maximal stimulation of α-adrenergic and endothelin receptors, free IP₃ in cardiac myocytes increased to ~30 nM [11]. While such biosensors are useful for detecting genuine increases in IP₃ concentration, they are less helpful in revealing the kinetics of IP₃ turnover, since binding of IP₃ 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 IP₃ concentration from a basal value of ~15 nM to a maximal value of ~35 nM within ~400 s, after which the IP₃ 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 PIP₂ and inositol phosphate levels drop dramatically during the isolation procedure [13,14].

Because IP₃ is hydrophilic, it translocates from the sarcolemma to the cytoplasm upon formation from PIP₂. The main cytoplasmic target of IP₃ is IP₃Rs in the membrane of the ER/SR. There are three IP₃R isoforms, denoted type 1, type 2 and type 3. Current evidence suggests that the heart expresses all three IP₃R isoforms, although there is some inconsistency in the literature regarding their relative ratios in different cardiac cells. It has been proposed that type 1 IP₃Rs 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 IP₃R and, to a lesser extent, type 3 IP₃R (e.g. [17,18]). Overall, the majority of studies have concluded that the most prevalent IP₃R isoform within contractile cardiomyocytes is type 2 (see Table 1). The few reports describing type 1 IP₃Rs being more abundant [15,16,19] could indicate differences in IP₃R expression between species, or that the probes employed lacked specificity.

The IP₃R isoforms vary with respect to IP₃ affinity, activation and inactivation by Ca²⁺, regulation by ATP, phosphorylation and other modulatory factors (e.g. [20,21]). It is believed that subtle variations in the sensitivity of the three IP₃R isoforms to such allosteric regulators can modulate the biophysical properties of the channels. Thus, depending on the expression and subcellular distribution of particular IP₃R isoforms, cells may respond to alterations in IP₃, Ca²⁺ and other modulators quite differently. Furthermore, IP₃Rs form macromolecular signaling complexes with other proteins including ankyrin B, chromogranin A and B, cytochrome *c*, ERp44, Homer, IP₃R-binding protein released with IP₃ (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 IP₃R opening, with consequent alteration of cellular Ca²⁺ signals (e.g. [25,26]). Although most of the data exploring IP₃R-binding proteins has been derived from studies of neuronal IP₃Rs, it is likely that cardiac IP₃Rs form similar macromolecular complexes. As yet, only Ca²⁺- and calmodulin-dependent protein kinase II (CaMKII) has been identified as an accessory protein of cardiac IP₃Rs [18].

An intriguing aspect of IP₃ 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 PIP₂-PLC-IP₃ 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 IP₃. Alternatively, given that IP₃ is highly diffusible inside cells [29], the IP₃ produced at the plasma membrane may be sufficient for activation of IP₃Rs throughout a cell. The particular interest in cardiac nuclear IP₃ signaling derives from a number of recent studies that have demonstrated expression of IP₃Rs within or around the nucleus in cardiac myocytes [18], and involvement of perinuclear Ca²⁺ 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 PIP₂-PLC-IP₃ 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 IP₃ may be signaling molecules in their own right. In particular, IP₄, IP₅ and IP₆ have been implicated in the regulation of nuclear functions (gene expression, chromatin remodeling, mRNA export and RNA editing). This adds to the complexity of IP₃ signaling and suggests that the potential roles of IP₃ go beyond the release of Ca²⁺ from internal stores. Higher inositol polyphosphates, including IP₄, IP₅ and IP₆, have been detected in the heart [31], as well as high affinity binding

sites for IP₄ and IP₆ [32,33]. However, their physiological functions in cardiac myocytes remain poorly understood.

3. Tools to study cardiac IP₃ signaling

Numerous tools are available to study cellular IP₃ signaling. They can be grouped into (A) methods to measure intracellular IP₃ levels, (B) pharmacological tools to manipulate IP₃ levels, IP₃ production and degradation, and the IP₃ receptor, and most recently (C) approaches that involve genetic manipulation of the intracellular IP₃ signaling cascade and the generation of transgenic animals with an altered IP₃ second messenger system.

(A) Of great interest for many years has been the quantitative determination of intracellular IP₃ levels. Until very recently, the real-time study of IP₃ 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 IP₃ precursors and degradation products. While these methods have generated important insights into cellular IP₃ dynamics, they are rather limited in deciphering the spatiotemporal organization of the IP₃ second messenger system at the cellular and subcellular level. Recent developments, however, have seen the arrival of novel fluorescent probes to study intracellular IP₃ dynamics. For example, a biosensor derived from the IP₃ binding domain of type-3 IP₃R, termed 'LIBRA', was employed to measure IP₃ concentrations in living cells [38]. Similarly, the intracellular translocation of a pleckstrin homology domain from phospholipase C_{δ1} fused to GFP was used to estimate [IP₃] [39], and to measure agonist-induced oscillatory changes of IP₃ concentration [40]. A recent study reported the construction and characterization of prototypic fluorescent biosensors that allow quantitative measurements of cellular IP₃ levels in a living cell with temporal and spatial resolution [11]. These sensors (termed FIRE-1 and -3, for 'Fluorescent IP₃ Responsive Element' type-1 and -3) utilized the IP₃R 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 IP₃. These constructs have been successfully employed to measure [IP₃] 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 IP₃ concentration, with free IP₃ levels rising to 30 nM. A point source of IP₃, experimentally achieved by membrane rupture with a patch pipette containing IP₃, allowed detailed spatiotemporal monitoring of intracellular IP₃ diffusion, revealing that IP₃ diffusion into the nucleus occurred with a delay, and that the elevation of nuclear IP₃ concentration had a blunted rise compared to that in the cytoplasm. Furthermore, stimulation with endothelin-1 (100 nM) caused [IP₃] 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 [IP₃] was 70±9% 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 IP₃ dynamics at high temporal and spatial resolution in intact living cells, although their potential to buffer IP₃ has to be taken into account.

(B) More conventional, but nonetheless invaluable tools for the study of IP₃ signaling are pharmacological agents that interfere with the IP₃ signaling cascade. Some commonly used reagents are blockers of IP₃ formation (e.g. the PLC inhibitor U73122 and its negative control U73343), or antagonists of the IP₃ 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 IP₃R agonists [44] and IP₃ species that can be activated photolytically [45,46] have further expanded the repertoire of tools for studying IP₃ signaling.

(C) Molecular technologies are also beginning to be employed to explore cardiac IP₃ function. In particular, the generation of animals or cells with genetically altered IP₃ 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 IP₃ affinity trap (which consisted of the ligand-binding domain of the rat type-1 IP₃R; also known as an 'IP₃ sponge') has been successfully used to abolish IP₃-evoked effects on Ca²⁺ signaling in rabbit ventricular myocytes [41]. The IP₃ sponge represents an experimental tool that can complement or replace pharmacological IP₃R blockers, and specifically attenuate IP₃ signaling. Potentially even better than the IP₃ sponge would be the introduction of an IP₃ metabolizing enzyme (e.g. the IP₃ 5' phosphatase), which does not necessarily have to be present at 1:1 stoichiometry to completely clamp IP₃ 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 IP₃R type-2 isoform [17]. This loss of response to endothelin-1 was specifically coupled to the absence of IP₃Rs, since the myocytes retained a normal positive inotropic response to β -adrenergic stimulation.

4. The dilemma of cardiac IP₃ receptors: lost in an ocean of ryanodine receptors

The evidence for IP₃R expression in the heart is indisputable. Numerous laboratories using a variety of techniques have established that cardiac myocytes possess these Ca²⁺ channels. Indeed, the notion that IP₃ mediates Ca²⁺ release in the heart is not new, and has been established by studies spanning the last two decades. The very earliest studies on cardiac IP₃ signaling found evidence for functional IP₃Rs capable of releasing Ca²⁺ from the SR [47–51]. However, compared with Ca²⁺-induced Ca²⁺ release through RyRs, though, the IP₃-induced Ca²⁺ release was slow and small. These data indicated that a major contribution of IP₃-induced Ca²⁺ release to cardiac excitation–contraction coupling was highly unlikely. As a result, IP₃Rs have been largely overlooked, and often regarded as making a minor contribution, if any, to cardiac Ca²⁺ signaling.

Cardiac excitation–contraction coupling relies principally on Ca²⁺-induced Ca²⁺ release through type 2 RyRs. Expression of RyRs, as the major SR Ca²⁺ 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 IP₃Rs in ventricular myocytes revealed that RyRs outnumber IP₃Rs 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 IP₃Rs. 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]. IP₃R 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 IP₃Rs than RyRs in ventricular SR. These data indicate that cardiac IP₃Rs face a serious problem; they have to generate a detectable Ca²⁺ signal against a large background of RyR-induced Ca²⁺ release. How can so few IP₃Rs have an impact on cardiac Ca²⁺ signaling, which is clearly dominated by RyRs? Or, put another way, how can cardiac myocytes decipher and make use of a small hormone-evoked IP₃-induced Ca²⁺ signal amidst the large action potential-evoked RyR-induced Ca²⁺ 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_3 Rs 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_3 Rs to be activated by Ca^{2+} [23]. The need for synergistic binding of both IP_3 and Ca^{2+} explains why IP_3 Rs 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_3 Rs 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_3 Rs 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_3 Rs 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_3 Rs, 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_3 Rs. 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_3 Rs, 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 IP₃ effects on cellular Ca²⁺ fluxes in a more quantitative way, which will help gain more insight into the actions of IP₃ on cellular Ca²⁺ homeostasis.

6. Elementary IP₃-induced Ca²⁺ release events in cardiac myocytes

Discrete, non-propagating elementary Ca²⁺ release events have been characterized in a variety of excitable and non-excitable cell types (for reviews see [73,74]), for both RyRs ('Ca²⁺ sparks'; [75–78]) and IP₃Rs [70,79,80]. Ca²⁺ sparks represent Ca²⁺ release from a restricted number of clustered RyRs opening in concert [81]. It is well established that Ca²⁺ sparks represent the elementary Ca²⁺ release events that spatially and temporally summate to produce the global Ca²⁺ transient during cardiac excitation–contraction coupling.

IP₃R-dependent elementary Ca²⁺ release events, termed 'Ca²⁺ blips' (single channel event) and 'Ca²⁺ puffs' (multi-channel event originating from a group of IP₃Rs), have been observed in non-excitable cells such as oocytes [79], HeLa [82] and vascular endothelial cells [80]. Ca²⁺ puffs differ from RyR-mediated Ca²⁺ sparks in having significantly slower kinetics. Despite the growing evidence that IP₃R-evoked Ca²⁺ release occurs in cardiac cells, reports of elementary Ca²⁺ signals arising from IP₃Rs in cardiac myocytes have been scarce. This is likely related to the significantly lower density of IP₃Rs, which practically means that Ca²⁺ puffs are difficult to discern in the 'Ca²⁺ noise' from RyR-dominated Ca²⁺ release. A recent report showed that this problem could be circumvented experimentally by eliminating Ca²⁺ release via RyRs pharmacologically [70]. This is illustrated in Fig. 1. Permeabilized cat atrial myocytes displaying spontaneous Ca²⁺ release activity were treated with the RyR inhibitor tetracaine [83], to block Ca²⁺ sparks. The cells were then exposed to IP₃, which resulted in the appearance of localized non-propagating Ca²⁺ elevations that were completely abolished by the IP₃R blocker heparin. In comparison to typical Ca²⁺ sparks, the IP₃-evoked Ca²⁺ 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 IP₃R-mediated elementary events did not differ significantly (for average numbers see Fig. 1D). However, the underlying Ca²⁺ release flux was clearly smaller for Ca²⁺ puffs compared to Ca²⁺ sparks and the declining phase of [Ca²⁺] was prolonged (Fig. 1C). In summary, the IP₃-dependent elementary Ca²⁺ release events in permeabilized atrial myocytes were distinctly different from Ca²⁺ sparks and were reminiscent of Ca²⁺ puffs typically observed in non-excitable tissue where IP₃-dependent Ca²⁺ signaling is predominant [79,80,82,84]. These data are consistent with the notion that IP₃Rs provide a significantly smaller Ca²⁺ release pathway compared to RyRs. However, their relative paucity does not eliminate their potential to affect cardiac Ca²⁺ signaling. It is plausible that IP₃R-dependent Ca²⁺ puffs can summate with RyR-mediated Ca²⁺ sparks to increase the amplitudes of electrically-evoked Ca²⁺ transients. In addition, Ca²⁺ puffs may activate neighboring RyRs via Ca²⁺-induced Ca²⁺ release and thereby amplify SR Ca²⁺ release. Alternatively, IP₃-mediated Ca²⁺ 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. IP₃ 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 Ca²⁺ 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 IP₃Rs may play a significant role in the development of the heart by contributing to the initiation of pacemaking activity, and thereby promote cardiogenesis. Spontaneous Ca²⁺ oscillations begin as soon as the heart tube is created. These Ca²⁺ 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 $\text{Na}^+/\text{Ca}^{2+}$ 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 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, pros-taglandins, IGF-1) with IP₃ production and Ca²⁺ 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 IP₃R types 1, 2 and 3 are expressed in neonatal cells [102,103,106].

A consistent message from the investigations of neonatal myocytes is that IP₃ causes perinuclear Ca²⁺ release. Almost all studies that examined either the distribution of IP₃Rs, or the spatial pattern of IP₃-evoked Ca²⁺ 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 IP₃-sensitive Ca²⁺ stores can impact specifically on nucleoplasmic Ca²⁺ 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 IP₃Rs 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 Ca²⁺-handling systems are put into place. However, IP₃Rs 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 IP₃Rs are viable and do not have any apparent cardiac defects [17]. Furthermore, complete single deletion of type 1, type 2 or type 3 IP₃Rs in mice, or combined knockout of types 2 and 3 IP₃Rs, does not prevent the murine heart (or whole animal) from developing [110,111]. These data could indicate that IP₃Rs are not essential for cardiogenesis. However, it is possible that there is functional redundancy between IP₃R isoforms, such that the presence of any one of the three isoforms will do. Alternatively, another Ca²⁺ signaling mechanism could compensate for the normally undertaken by IP₃Rs. Although knockout of types 2 and 3 IP₃Rs appears to be benign, mice not expressing type 1 IP₃Rs 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. IP₃ signaling in atrial myocytes

There is substantial evidence for IP₃-mediated Ca²⁺ signaling in atrial cells. The atrial myocardium expresses numerous receptors coupling to the PIP₂-PLC-IP₃ cascade. Increased phosphoinositide breakdown and inositol phosphate generation in atrium is mediated by receptors for acetylcholine (muscarinic, M₁ and M₃), angiotensin II (AT₁), endothelin (ET_A), histamine (H₁), 5-hydroxytryptamine (5-HT₂), norepinephrine (α₁) and vasopressin in various species including cat, chick, guinea-pig, mouse, rat and human [10,112–125]. Furthermore, expression of IP₃Rs 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 IP₃Rs and may also express type 3 IP₃Rs [41]. Human atrial myocytes, on the other hand, have been suggested to express predominantly type 1 IP₃R [15], although this requires further clarification.

Early evidence for an involvement of IP₃ 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 IP₃ to permeabilized atrial muscle [50], photorelease of IP₃ [126] and, more recently, exposure of isolated atrial myocytes to a membrane permeable analogue of IP₃ [58,63,127,128] provided definitive proof that IP₃ induces a positive inotropic effect in atrium. Thus, an important action of IP₃ in atrium is to increase the force of contraction.

Interestingly, receptor-mediated inositol phosphate production and expression of IP₃Rs 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 IP₃Rs 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, IP₃R density amounted to 1.8 pmol/mg protein in rat atrium [58]. Thus, atrial SR contains more IP₃Rs, but less RyRs, than ventricular SR, suggesting that SR Ca²⁺ release in atrial myocytes is more dependent on IP₃ signaling than in ventricular myocytes. In line with this notion, direct application of IP₃ to permeabilized rat myocytes revealed a ~5 times larger global Ca²⁺ 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 PIP₂-PLC-IP₃ 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]).

IP₃ does not alter myofilament Ca²⁺ responsiveness [48]. Rather, the positive inotropic effect of IP₃ in atrial myocardium is mediated by enhanced Ca²⁺ release from the SR. The extra component of Ca²⁺ mobilization from IP₃Rs adds to the action potential-induced RyR-mediated Ca²⁺ release, and thereby increases the Ca²⁺ transient underlying contraction. Potentiation of Ca²⁺ transients following activation of IP₃Rs has been observed during stimulation of cat, mouse, rabbit and rat atrial myocytes with endothelin-1 or a membrane-permeant form of IP₃ (IP₃BM) [17,63,70–72,127,128]. The increase in the electrically-stimulated Ca²⁺ transient occurred without alteration of the Ca²⁺ load within the SR [72]. Thus, IP₃ increased fractional Ca²⁺ release from the SR (by ~13%) and did not affect SR Ca²⁺ storage per se [17,72].

IP₃-induced Ca²⁺ release may also lead to arrhythmogenic alterations in atrial Ca²⁺ signaling. In cat and rabbit atrial myocytes, endothelin-1 induced Ca²⁺ alternans [70,132], which may degenerate into arrhythmogenic Ca²⁺ waves [133]. Furthermore, endothelin-1 or IP₃BM elicited spontaneous Ca²⁺ release events during diastole in cat, mouse, rabbit and rat atrial myocytes. These spontaneous signals ranged from spark-like events and Ca²⁺ waves to extra action potential-derived global Ca²⁺ transients [63,70,71,128,132]. Similarly, in human atrial myocardium, endothelin-1 induced arrhythmic extra contractions mediated by activation of PLC and IP₃Rs [134,135] and in isolated human atrial myocytes angiotensin II, another agonist coupling to PLC-IP₃ signaling, increased the frequency of spontaneous Ca²⁺ sparks without altering SR Ca²⁺ load [136].

IP₃-induced arrhythmogenic Ca²⁺ release likely involves the recruitment of RyRs via Ca²⁺-induced Ca²⁺ release. In rat atrial myocytes, some IP₃Rs and RyRs are colocalized in the subsarcolemmal area closely apposed to the sarcolemmal Na⁺/Ca²⁺ exchanger (NCX), as illustrated in Fig. 2. The proximity of the two types of Ca²⁺ channels makes it likely that activation of IP₃Rs will provoke RyR opening. The consequent Ca²⁺ signal would then activate the nearby NCX, thereby generating a depolarizing current. Recruitment of multiple IP₃R/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 Ca²⁺ transient. In support of this scheme, it has been demonstrated that the spontaneous diastolic action potential-evoked Ca²⁺ transients observed during endothelin-1 or IP₃BM application are preceded by subsarcolemmal Ca²⁺ release events (probably IP₃R-activated Ca²⁺ sparks; [63]). Furthermore, in human atrial myocardium, inhibition of RyRs suppressed endothelin-1-induced arrhythmias. These data suggest that the relatively few IP₃Rs in the atrium can cause a disproportionately large Ca²⁺ 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 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 IP₃Rs would be to act as an inotropic support for the ventricles by boosting the amplitude of systolic Ca²⁺ transients, and thereby increasing contractile force. Consistent with this notion, IP₃Rs have been localized close to the dyadic junctions where excitation–contraction coupling is initiated [142], and direct activation of IP₃Rs was found to enhance Ca²⁺ spark occurrence throughout ventricular cells [41]. Furthermore, application of hormones that activate the production of IP₃ inside ventricular myocytes, such as endothelin-1 [11], enhances systolic Ca²⁺ signals in rat and rabbit cells [41,128,143]. IP₃ 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 IP₃R 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 IP₃Rs in ventricular myocytes have concluded that the predominant effect is to stimulate arrhythmias [62,143]. Indeed, IP₃R 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 Ca²⁺ flux through the relatively few IP₃Rs 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 IP₃Rs in ventricular myocytes is their localization close to the nucleus. A substantial proportion of IP₃Rs 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 IP₃ can release Ca²⁺ 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 IP₃Rs potentially allows the generation of autonomous nuclear Ca²⁺ 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 IP₃-sensitive Ca²⁺ stores promotes a CaMKII-dependent phosphorylation of histone deacetylase 5 (HDAC5) thereby causing it to be exported out of the nucleus [140]. Thus, perinuclear IP₃-evoked Ca²⁺ 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 IP₃ 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 Ca²⁺-dependent gene transcription inside cells that experience periodic surges of Ca²⁺ during excitation–contraction coupling [155]. Perinuclear IP₃Rs may provide a resolution to this conundrum by providing a source of Ca²⁺ 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 IP₃Rs in rat neonatal ventricular myocytes using a membrane-permeant IP₃ analogue or an α₁-adrenergic agonist causes perinuclear Ca²⁺ release events, and that blocking IP₃R 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 IP₃Rs triggered nucleoplasmic Ca²⁺ signals and hypertrophic gene transcription [157]. Further work is required to establish whether this scheme is relevant *in vivo*. However, the concept that IP₃Rs impact specifically on nuclear Ca²⁺ signaling is gaining momentum. Numerous recent reports examining Ca²⁺ signaling in neonatal [103,108,109,157], atrial [71,72,137] and ventricular myocytes [140] have demonstrated specific effects of IP₃-generating agonists or IP₃ itself on nucleoplasmic Ca²⁺ signals. Coupling these data with the observations that IP₃ is necessary for some forms of hypertrophy [109,157,158] raises the possibility that IP₃Rs are key regulators of cardiac myocyte gene transcription and fate.

10. IP₃Rs in other cardiac cells

The majority of studies examining cardiac IP₃R expression and function have focused on developing embryonic/neonatal cells, or adult atrial and ventricular myocytes. However, there are reports suggesting that IP₃Rs are also expressed in other cardiac regions/cell types, such as papillary muscle [48,159]. It has also been proposed that IP₃Rs 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 IP₃, and thereby prevents its Ca²⁺-mobilising action, or an anti-IP₃R 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 Ca²⁺ release to activate depolarizing NCX current, which contributes to diastolic depolarization and pacemaker activity (for review see [160]). This 'intracellular Ca²⁺ clock' is fueled by high, cAMP-dependent Ca²⁺ 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 Ca²⁺ load and release). Because of this Ca²⁺ dependence of pacemaker activity, it appears feasible that IP₃R-mediated SR Ca²⁺ release might also modulate pacemaker activity in myocytes from adult sinus node. Direct evidence for an involvement of IP₃ 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 IP₃ in supraventricular tissue, exert positive chronotropic effects in mammalian heart via activation of AT₁ and ET_B receptors, respectively (e.g. [161,162]). Whether PLC-IP₃ 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-IP₃ signaling and IP₃R-mediated SR Ca²⁺ release depolarize the membrane potential of pacemaker cells thereby increasing heart rate [163].

Purkinje cells also express IP₃Rs [164], seemingly to a greater extent than either atrial or ventricular myocytes [16]. The physiological function of IP₃Rs 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 Ca²⁺ waves that are reduced in frequency and amplitude by 2-APB. Also consistent with the notion of IP₃Rs regulating automaticity of Purkinje fibers, reduction of IP₃ production using a phospholipase C inhibitor was demonstrated to inhibit their autonomous Ca²⁺ spiking [102].

An overview on the expression of IP₃R isoforms in various regions of adult myocardium and the involvement of IP₃ 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 IP₃ expression in various cardiac diseases (for a more detailed discussion on this issue see below). It should be noted that, unlike RyR2, IP₃Rs 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 IP₃Rs and alterations thereof in cardiac disease should be confirmed in cardiac myocytes. Otherwise, the possibility exists that the findings are obscured by IP₃R expression from non-myocytes. For example, rat heart contains all three IP₃R isoforms with the IP₃R type 1 being the most abundant isoform (more than 60% of total IP₃R mRNA). Ventricular myocytes, however, do not contain IP₃R type 1 (less than 2% of total IP₃R mRNA), but rather express the type 2 (85%) and 3 (14%) IP₃R [57]. Thus, we have also indicated in Table 1 whether or not IP₃R 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 $\text{CaMKII}\delta_B$ 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 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, hyper-trophy 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 IP₃ concentration in cardiac myocytes. The transient increase in IP₃ 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 IP₃ concentration correlates with the induction of arrhythmias, ventricular tachycardia and ventricular fibrillation [145]. Substances able to inhibit the IP₃ 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 Ca²⁺ influx through reverse mode NCX leads to an increase in SR Ca²⁺ load. IP₃-induced SR Ca²⁺ release with subsequent activation of forward mode NCX may then trigger the arrhythmias. Reperfusion-induced increase in IP₃ 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 IP₃R in cardiac myocytes. High affinity interaction between ankyrin-B and IP₃R is required for post-translational stability and targeting of the IP₃R to SR microdomains closely opposed to neighboring T tubules [178,179]. Loss-of-function mutations in ankyrin-B cause reduction of IP₃Rs 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 syncope and sudden death. The cellular mechanisms underlying the ankyrin-B-related arrhythmias may involve altered Na⁺ and Ca²⁺ homeostasis. The macromolecular complex in the SR–T tubule junctions is composed of ankyrin-B, IP₃R 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 [Ca²⁺] levels and unaltered L-type Ca²⁺ currents but increased Ca²⁺ transients and an increased propensity toward catecholamine-induced arrhythmogenic SR Ca²⁺ release [142] pointing to SR Ca²⁺ overload as a causative factor. It has been suggested that IP₃Rs act as “Ca²⁺ pressure valves” releasing Ca²⁺ 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 IP₃R for cardiac function.

An interesting feature of the above hypothesis is that loss-of-function of IP₃Rs (or mis-targeting) appears to be responsible for the ankyrin-B-related arrhythmias, which may ultimately be triggered by RyR-mediated SR Ca²⁺ release due to SR Ca²⁺ overload. This is in striking contrast to the arrhythmias triggered directly by activation of IP₃Rs and IP₃R-induced SR Ca²⁺ release at normal SR Ca²⁺ load. Thus, both activation and loss-of-function of IP₃Rs 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. Ca²⁺ homeostasis is altered in myocytes from diabetic hearts such that electrically stimulated Ca²⁺ transients are reduced and prolonged. These alterations are the result of changes in the expression and function of SR Ca²⁺ handling proteins; SERCA2a, NCX and RyR expressions are reduced, whereas phospholamban expression is increased and its phosphorylation decreased. As a consequence, SR Ca²⁺ load is reduced, and SR Ca²⁺ release and re-uptake are impaired [181,182]. Interestingly, an increased

propensity toward arrhythmogenic afterdepolarizations has been observed in diabetic cardiomyocytes [182]. Insulin increases IP₃ concentration and triggers arrhythmic Ca²⁺ release events in myocytes from diabetic *ob/ob* mice – but not from wild-type mice – and this effect is mediated via IP₃ signaling [183]. IP₃R expression (type 1 and type 2 IP₃Rs) 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 IP₃ production in response to α -adrenergic stimulation has been reported in diabetes [186,187]. PIP₂ levels and the expression and activities of PLC isoenzymes are reduced [188]. Taken together, these data indicate that altered receptor-PLC coupling, IP₃ generation and IP₃-induced SR Ca²⁺ release may contribute to impaired Ca²⁺ 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 AT₁, 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 IP₃ 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 IP₃ signaling in hypertrophy may be unaltered or even impaired [189,190]. More recent studies, however, collectively point toward an important role of PLC-IP₃ signaling in the development and progression of hypertrophy. For example, in pressure overload-induced hypertrophy, PLC activity was elevated and norepinephrine-induced generation of IP₃ was increased [191]. Moreover, IP₃-induced Ca²⁺ release from the SR was larger in myocytes from hypertensive rats than from control rats [192]. In volume overload-induced hypertrophy, sarcolemmal PIP₂ levels were decreased, whereas PLC β 1 and PLC γ 1 expression and activities and IP₃ levels were increased [193]. Furthermore, in hypertrophy induced by transient overexpression of a constitutively active G_q α , PLC β 1 and PLC β 3 expressions were elevated [194]. Thus, PLC-IP₃ signaling and IP₃-induced Ca²⁺ 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 IP₃-induced Ca²⁺ release appears to contribute to increased expression of the very machinery responsible for the generation of IP₃, 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 IP₃ 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], AT₁ receptors are down-regulated (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 PIP₂, PI-4 kinase, PIP-5 kinase, PLC isoforms and inositol phosphates [200,201]. Expression of IP₃Rs is increased in both human and animal heart failure, whereas RyR levels are reduced, indicating a shift toward more IP₃R-mediated Ca²⁺ 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 IP₃ formation as well as in the regulation of IP₃Rs suggesting that IP₃-induced Ca²⁺ 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 IP₃ signaling in cardiac myocytes. There is no longer any doubt that IP₃ 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 IP₃ signaling in adult cardiac myocytes is that apparently it exerts more adverse than beneficial effects. Is IP₃ 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 IP₃R isoforms? How is expression of IP₃Rs regulated? What is the composition, subcellular distribution and functional role of IP₃R macromolecular complexes in cardiac myocytes? Which kinases and phosphatases regulate cardiac IP₃R phosphorylation and what is the functional impact of this regulation? By what other means is IP₃R function regulated? How exactly is IP₃ signaling involved in the development and progression of cardiac diseases? Future studies should aim at answering these questions. Clearly, IP₃ 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.

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Fig. 1.

Elementary IP₃-induced Ca²⁺ release events in cardiac myocytes. (A) Confocal linescan image of Ca²⁺ sparks in a permeabilized cat atrial myocyte (control). Tetracaine (Tetrac) abolishes Ca²⁺ sparks. Addition of IP₃ elevates baseline Ca²⁺ concentration and causes the appearance of smaller Ca²⁺ release events, i.e. Ca²⁺ puffs, which are suppressed by heparin. (B) Averaged Ca²⁺ spark and Ca²⁺ puff. (C) Normalized Ca²⁺ spark (black) and Ca²⁺ puff (red) (top) and first derivative thereof (bottom), a measure for the underlying Ca²⁺ release flux. The Ca²⁺ puff exhibits slower kinetics and lower Ca²⁺ release flux than the Ca²⁺ spark. (D) Surface plots of averaged linescan images of Ca²⁺ sparks and Ca²⁺ puffs. The Ca²⁺ puff displays lower amplitude, longer rise time and duration, and similar width as compared to the Ca²⁺ spark. Modified from [70]. Copyright of the Journal of Physiology; used by kind permission.

Fig. 2.

Colocalization of subsarcolemmal IP₃Rs and RyRs in atrial myocytes. (A) Confocal image of a rat atrial myocyte immunostained for the expression of IP₃Rs (red) and RyRs (green). N denotes the nucleus. The area marked by the dashed white rectangular is shown enlarged in (B). Colocalization of IP₃Rs and RyRs (yellow) occurs in some areas underneath the sarcolemma. (C) Schematic illustration of colocalization of subsarcolemmal IP₃Rs 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.

Fig. 4.

Overview on IP₃ 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 IP₃ concentration via activation of phospholipase C (PLC). IP₃Rs 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. IP₃ signaling is involved in excitation–contraction coupling, in excitation–transcription coupling and in the generation of arrhythmias. Excitation–contraction coupling is mediated by Ca²⁺-induced Ca²⁺ release (CICR) from the SR through RyR2. Cytoplasmic Ca²⁺ binds to the myofilaments and initiates contraction. IP₃-induced Ca²⁺ release from the SR through IP₃Rs may contribute to excitation–contraction coupling by increasing the Ca²⁺ transient and thus contraction. On the other hand, IP₃-induced Ca²⁺ release from (subsarcolemmal) SR may trigger further Ca²⁺ release through neighboring RyR2 with subsequent activation of sarcolemmal NCX. This induces arrhythmias via generation of delayed afterdepolarizations and triggered activity. IP₃-induced SR Ca²⁺ release also generates pro-arrhythmic Ca²⁺ alternans. Finally, IP₃ is involved in excitation–transcription coupling via modulation of nuclear Ca²⁺ concentration. Nuclear Ca²⁺ may be increased by IP₃ in three ways: (1) IP₃ may increase the cytoplasmic Ca²⁺ transient. Cytoplasmic Ca²⁺ then diffuses through the nuclear pores into the nucleoplasm to elicit a delayed nuclear Ca²⁺ transient. (2) Cytoplasmic IP₃ may itself diffuse through the nuclear pores into the nucleus to activate nuclear IP₃Rs facing the nucleoplasm. Ca²⁺ release from the nuclear envelope, a Ca²⁺ store continuous with the SR, into the nucleoplasm then increases the nuclear Ca²⁺ concentration directly. (3) Finally, IP₃ may also activate IP₃Rs on the nuclear envelope facing the cytoplasm. Ca²⁺ release from the nuclear envelope into the cytoplasm may then increase nuclear Ca²⁺ 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, $\text{Na}^+/\text{Ca}^{2+}$ exchanger; PLC, phospholipase C.

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

Table 1

Expression of IP3R isoforms in adult myocardium

Species	IP ₃ R isoform	Specimen	Methods	Confirmed in myocytes?	Findings	Reference
Bovine	1	A, V, CS	WB, IHC, IP ₃ binding	Yes	IP ₃ R1: P > AV >> A, V	[16]
Canine	1, 2	LA tissue, myocytes	RT-PCR, WB, IHC	Yes	IP ₃ R1 in cyto; IP ₃ R2 in cyto, NE, ID; IP ₃ R1,2 up in AF	[170]
Ferret, Rat	1, 2, 3	Heart, V myocytes	RNA analyses, IP ₃ binding, IP ₃ R reconstitution	Yes (IP ₃ R2,3)	IP ₃ R1 predominant in myocardium, but absent in myocytes; IP ₃ R2,3 predominant in myocytes (85% vs. 14%)	[57]
Human	1	LV, RV, Septum	NB, <i>in situ</i> hybridization	Yes	IP ₃ R1 up in HF	[19]
Human	1	RA tissue	RT-PCR, WB, IHC	Yes	IP ₃ R1 in cyto, NE; IP ₃ R1 up in AF	[15]
Human	1	RA tissue	RT-PCR	No	IP ₃ R1 up in AF	[169]
Human	1, 2, 3	RA tissue	RT-PCR	No	IP ₃ R1,2,3 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 ₃ R2 >> IP ₃ R1,3; IP ₃ R2: A>V	[17]
Rabbit	2	LV myocytes	WB	Yes	IP ₃ R2 up in HF	[202]
Rabbit	2, 3	A, V myocytes	WB	Yes	IP ₃ R2,3: A>V	[41]
Rat	n.d.	Heart, A, V	WB, IHC, EM	Yes	IP ₃ R in ID in A, V	[203]
Rat	n.d.	Heart, A, V	RNA analyses, WB, IHC, <i>in situ</i> hybridization	Yes	IP ₃ R expression in cardiomyocytes (RyR:IP ₃ R ≈ 50:1)	[54]
Rat	n.d.	Heart, A, V, CS	RNA analyses, <i>in situ</i> hybridization	Yes	IP ₃ R: CS >A=V; Senescence: A>V	[204]
Rat	1, 2, 3	A, V, Purkinje	RT-PCR, WB, ICC, IP ₃ binding	Yes (IP ₃ R2)	A>V; IP ₃ R2 in A myocytes in subsarcolemmal SR	[58]
Rat	2	A myocytes	ICC	Yes	IP ₃ R2 colocalizes with RyR in subsarcolemmal SR	[63]
Rat	1,2	A, V	RT-PCR WB	No	IP ₃ R1,2: LA > RA >> LV=RV; IP ₃ R1,2 up by stress in glucocorticoid-dependent manner	[138]

Species	IP ₃ R isoform	Specimen	Methods	Confirmed in myocytes?	Findings	Reference
Rat	2	Heart (nuclear fraction), V myocytes	WB, ICC	Yes	IP ₃ R2 predominant in NE	[18]
Rat	1	LA, LV	RT-PCR, WB	No	IP ₃ R1 down after depletion of catecholamines	[205]
Rat	2	Heart	RT-PCR, WB	No	IP ₃ R2 down in diabetes	[185]
Rat	1, 2, 3	LA, LV, RA, RV	RT-PCR, WB	No	IP ₃ R1,2 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⁺/Ca²⁺ 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.