Review

Ins(1,4,5)P₃ and cardiac dysfunction

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1. Introduction

Cardiac related death occurs rapidly via the development of arrhythmias or more slowly by a progressive decline in mechanical performance reflecting decreased contractile activity as well as loss of viable myocytes. Pathological conditions such as myocardial ischaemia, inflammatory heart diseases and various forms of heart failure are associated with both sudden death and also with a more progressive decrease in cardiac function. Electrophysiological disturbances leading to malignant ventricular arrhythmias are the usual cause of sudden cardiac death, whereas a gradual loss of functional myocytes, or a decline in their performance, underlies heart failure ultimately leading to cardiac decompensation. The mechanisms initiating these scenarios are complex and multifactorial, involving both extracellular and intracellular factors. However, given the continued major contribution of cardiac deaths to overall mortality, understanding the processes which initiate these pathological events is of prime importance. The current review will focus on changes in the intracellular milieu and in particular the potential role of the Ca²⁺-releasing second messenger inositol(1,4,5)trisphosphate [Ins(1,4,5)P₃] in initiating arrhythmias and cell death in cardiac pathologies.

Ins(1,4,5)P₃ is generated along with sn-1,2-diacylglycerol following the activation of either G protein-coupled receptors and phospholipase C-β (PLC-β) isoforms or receptor tyrosine kinases and PLC-γ isoforms [1]. The Ins(1,4,5)P₃, which is rapidly released into the cytoplasm, especially after activation of G protein-coupled receptors, activates Ins(1,4,5)P₃ receptors on Ca²⁺ stores in the endoplasmic reticulum [sarcoplasmic reticulum (SR) in muscle cells] causing a rapid and quantal release of Ca²⁺ [2]. In most non-cardiac cell types, this Ins(1,4,5)P₃-initiated Ca²⁺ response can be perpetuated by the process of Ca²⁺-induced Ca²⁺ release mediated by either the Ins(1,4,5)P₃ receptors or the related family of ryanodine receptors, the overall effect of this being that the Ca²⁺ signal within the cell is regulated spatially as well as quantitatively [3,4].

Instead of being primarily regulated by receptors coupled to Ins(1,4,5)P₃ generation, in heart the control of Ca²⁺ and hence the contractile state of the myocytes is predominantly regulated by the electrical activity of the sarcolemma. The cardiac action potential is initiated by depolarization of the sarcolemma and sustained in the plateau phase by the activation of voltage-gated L type Ca²⁺ channels (IᵥCaL). This in turn leads to a subsequent release of Ca²⁺ from the ryanodine receptor-operated stores of the SR. The Ca²⁺ released from the SR binds to troponin-C and activates the coupling of actin to myosin, resulting in contraction of myofilaments. Removal of Ca²⁺ from the cytosol is accomplished by means of several mechanisms including the sarcolemmal Na⁺–Ca²⁺ exchanger and the Ca²⁺-ATPases of the sarcolemma and the SR. None of these processes involve Ins(1,4,5)P₃ and factors which activate PLC, such as αₐ-adrenergic agonists and endothelin, are not major regulators of cardiac function under physiological conditions. In comparison with the cardiac ryanodine receptors, Ins(1,4,5)P₃ receptors in heart tissue respond only weakly to Ins(1,4,5)P₃ and release Ca²⁺ slowly [5]. It is probably this slow release which prevents the Ca²⁺ generated by Ins(1,4,5)P₃ from inducing Ca²⁺-induced Ca²⁺ release. Instead, the Ca²⁺ generated...
by Ins(1,4,5)γ has been reported to initiate slow Ca\(^{2+}\) oscillations [6]. Such slow increases in [Ca\(^{2+}\)], potentially have at least three acute effects on the cardiac action potential. First, it can stimulate a transient inward current (I\(_{in}\)) evoking delayed afterdepolarizations so that triggered activity can develop in otherwise quiescent ventricular muscle [7,8]. Second, the slowing of repolarization caused by elevated [Ca\(^{2+}\)], can favour conditions for reentry [9]. Third, it can cause intercellular uncoupling with conduction slowing [10–13]. Any of these alterations in myocardial electrical activity are potentially proarrhythmic and furthermore, Ins(1,4,5)P\(_3\) has been reported to activate Na\(^+-\)Ca\(^{2+}\) exchange, which might also be expected to increase the likelihood of arrhythmias, especially under ischaemic or reperfusion conditions [14].

In addition to these direct effects, elevated [Ca\(^{2+}\)], can affect myocyte function indirectly by activating a variety of intracellular components such as Ca\(^{2+}\)-dependent protein kinases, phosphatases, proteases and endonucleases [15,16]. Such actions are likely responsible for the more chronic effects of Ca\(^{2+}\) overload. Ins(1,4,5)P\(_3\) has been reported to be required for signalling pathways leading to apoptotic cell death in lymphoid cells [17,18] when apoptosis was induced by a number of different agents including ionizing radiations, Fas activation and glucocorticoids [19]. The requirement for functional Ins(1,4,5)P\(_3\) receptors for apoptotic signalling under these conditions points to a need either for elevated cytosolic Ca\(^{2+}\) or depletion of intracellular stores. However, not all apoptotic pathways appear to utilise intermediates which interact with Ca\(^{2+}\) stores. For recent reviews on signalling pathways in apoptosis see Refs. [20–23].

2. Inflammatory heart disease

Immunological mechanisms are involved in a variety of cardiac pathologies including heart transplant rejection, myocarditis (and the resulting dilated cardiomyopathy) as well as Chagas’ heart disease [24,25]. These diseases are characterised by lymphocyte infiltration, a decline in cardiac performance, due in part to loss of viable myocytes, and life-threatening arrhythmias. Many of these effects can be mimicked in isolated cardiomyocyte preparations. Addition of activated cytotoxic T lymphocytes to isolated cardiomyocytes caused the generation of electrophysiological disturbances, primarily prolongation of action potential duration, and associated early afterdepolarizations which were followed by Ca\(^{2+}\) overload, hypercontraction and cell death. These responses could be prevented either by using U-73122, an inhibitor of PLC, or by blocking the Ins(1,4,5)P\(_3\) receptor with heparin [26]. Cytocidal lymphocytes posses two distinct lytic mechanisms [27]. One of these involves perforins which create transmembrane pores allowing the entry of granzymes secreted by the lymphocytes. The second pathway involves the Fas ligand expressed on the surface of the lymphocytes binding to the Fas (receptor) (CD95/Apo-1) and activating apoptotic pathways similar to those initiated by TNF-α [20,28]. Using cytotoxic T lymphocytes from perforin knockout mice, activation of Fas was shown to account for perforin-independent myocyte damage. Direct activation of Fas caused [Ca\(^{2+}\)], overload associated with afterdepolarizations, followed by eventual cell death. Once again, inhibition of the Ins(1,4,5)P\(_3\) pathway prevented all of these changes [29]. In support of these findings, adding PLC to guinea-pig ventricular myocytes was shown to cause functional alterations resembling those seen in myocytes conjugated to cytotoxic T lymphocytes, including a reduction of the resting potential, induction of delayed afterdepolarizations, increased [Ca\(^{2+}\)], and cell destruction [30]. These studies pointed to Ins(1,4,5)P\(_3\) as a prime initiator of cardiomyocyte damage, being involved in the short term in the generation of arrhythmias, and with longer exposure in cell death pathways. The data also suggest that Fas activation causes an increase in either Ins(1,4,5)P\(_3\) content or in myocyte sensitivity to Ins(1,4,5)P\(_3\). To date, Fas has not been shown to directly cause generation of Ins(1,4,5)P\(_3\). It does not appear to bind PLC-γ nor to interact with G proteins to activate PLC-β isoforms [23]. However, addition of activating anti-Fas antibodies to cardiomyocytes caused Ins(1,4,5)P\(_3\)-dependent action potential disturbances that preceded hypercontraction and cell death [29]. This argues against a late increase in Ins(1,4,5)P\(_3\) following Ca\(^{2+}\) overload and apoptotic death. This scenario therefore suggests that Ins(1,4,5)P\(_3\) generation initiates changes in Ca\(^{2+}\) which, in turn, cause electrophysiological disturbances resulting in further Ca\(^{2+}\) overload and eventual cell dysfunction. More definitive evidence for this was supplied by experiments in which Ins(1,4,5)P\(_3\) was added to myocytes intracellularly and was shown to mimic the effects of Fas activation on action potential characteristics. Importantly, the inactive isomer, Ins(1,3,4)P\(_3\) did not cause either action potential changes or cell death [29]. These experiments and their findings are shown diagrammatically in Fig. 1.

The requirement for Ins(1,4,5)P\(_3\) receptor activation for Fas-induced cardiomyocyte death is reminiscent of earlier studies in lymphocytes. These studies reported resistance to apoptosis in cells deficient in the type-3 [17] or type-1 receptor [19]. The type-3 receptor in lymphocytes is largely plasmalemmal whereas the type-1 receptor resides on the endoplasmic reticulum. Thus, while implying a role for Ca\(^{2+}\) in the apoptotic response, these studies identified different mechanisms as being responsible for regulating the Ca\(^{2+}\) levels. However, a number of studies have reported a relationship between apoptosis and depletion of intracellular Ca\(^{2+}\) stores without a requirement for entry of extracellular Ca\(^{2+}\) [31,32]. While a number of studies have reported that the type-1 receptor predominates in heart [18,33,34], recent studies have presented evidence that the
Fig. 1. Initiation of electrophysiological disturbances (ΔV) and apoptosis by Fas activation in cardiomyocytes. Inhibition of \( \text{Ins}(1,4,5)P_3 \) generation by U-73122 or of its interaction with its receptor by heparin abolished the effects of Fas activation. In addition, direct intracellular application of \( \text{Ins}(1,4,5)P_3 \) but not \( \text{Ins}(1,3,4)P_2 \) mimicked the effects of Fas. The mechanisms by which Fas activates the \( \text{Ins}(1,4,5)P_3 \) response pathway are currently unknown.

Not all studies have demonstrated a requirement for disturbances in cytosolic Ca\(^{2+}\) or depletion of Ca\(^{2+}\) stores and why Ca\(^{2+}\) should be required for apoptosis under some conditions and not others is not clear. Some of the enzymes involved are Ca\(^{2+}\) requiring, especially the nucleases [36] and transglutaminases [37], but these are unlikely to be of central importance in the early stages of the response. The pathways involved in apoptotic death following Fas activation are diverse [22,23]. The death domain of activated Fas binds adapter proteins which in turn activate caspases (cysteine proteases). These are primarily responsible for the activation of nucleases and the destruction of important signalling and structural proteins. Activated Fas also signals to the stress-activated protein kinase, c-Jun N-terminal kinase, which can in turn initiate caspase activation [38]. Furthermore, Fas also causes the generation of ceramide, which has been reported to activate Ras, raising the possibility of initiating multiple signalling pathways [39]. The contributions of these various pathways to apoptotic and anti-apoptotic responses most likely vary between cell types and which are involved in Fas-mediated apoptosis in cardiomyocytes is currently unknown.

Another important question is whether the electrophysiological changes observed after activation of cardiomyocyte Fas receptors are required for apoptosis or whether these reflect two separate detrimental responses. \( \text{Ins}(1,4,5)P_3 \) has also been reported to be essential for apoptosis in non-excitable cells [17,19], suggesting that electrophysiological changes are not an absolute requirement for apoptosis. However, in heart electrophysiological disturbances might serve to further increase the Ca\(^{2+}\) overload and thus potentiate the cell death pathway. This possibility is illustrated by the broken arrow in Fig. 2.

### 3. Ischaemia and reperfusion

Reduction of the blood supply to the heart (ischaemia) and the re-introduction of flow (reperfusion) are associated with the development of arrhythmias [40,41] and with myocyte death, partly due to apoptosis [42]. Arrhythmias occurring acutely during ischaemia and reperfusion are often associated with an activation of the sympathetic

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![Diagram](image_url)
nervous system and there is evidence for linkage to stimulation of \( \alpha_1 \)-adrenergic receptors [40,43]. In addition, thrombin has been shown to be directly proarrhythmic under these pathological conditions by mechanisms unrelated to its coagulant activity [44,45]. The finding of proarrhythmic activity of \( \alpha_1 \)-adrenergic agonists and thrombin, only under ischaemic and reperfusion conditions, suggests some perturbation of the signalling pathways activated by \( \alpha_1 \)-receptors and thrombin receptors in the myocardium under these conditions.

Both \( \alpha_1 \)-adrenergic receptors and thrombin receptors in heart are coupled to phosphatidylinositol (PtdIns) turnover and thus their stimulation has the potential to generate \( \text{Ins}(1,4,5)\text{P}_3 \). While neither of these effectors causes rapid changes in \( \text{Ins}(1,4,5)\text{P}_3 \) content under physiological conditions, under conditions of early reperfusion, i.e., within 5 min of re-initiation of flow, large quantities of \( \text{Ins}(1,4,5)\text{P}_3 \) are generated in the presence of either \( \alpha_1 \)-receptor or thrombin receptor activation in hearts perfused with blood-free medium [45,46].

Two different experimental approaches suggest a relationship between the generation of \( \text{Ins}(1,4,5)\text{P}_3 \) and the development of reperfusion arrhythmias. First, there was a close correlation between the potencies of aminoglycosides and polyamines (which bind to the precursor lipid PtdIns(4,5)P_2) in inhibiting \( \text{Ins}(1,4,5)\text{P}_3 \) responses during reperfusion and their potencies in preventing the development of arrhythmias under these conditions [47]. Second, the \( \text{Ins}(1,4,5)\text{P}_3 \) and arrhythmogenic responses to thrombin were inhibited by the PLC inhibitor U-73122 whereas those initiated by stimulation of \( \alpha_1 \)-receptors were not [45]. The selectivity of the anti-arrhythmic activity of U-73122 to arrhythmias initiated by thrombin provided strong evidence that \( \text{Ins}(1,4,5)\text{P}_3 \) generation is necessary for the development of arrhythmias during early reperfusion (Fig. 3). It is of interest that in addition to preventing reperfusion arrhythmias, the aminoglycoside streptomycin has been shown to prevent arrhythmias caused by wall stress [48], a perturbation which results in generation of \( \text{Ins}(1,4,5)\text{P}_3 \) [49–52].

In addition to acute arrhythmias, reperfusion of ischaemic myocardium is also associated with substantial myocyte cell death, especially when longer reperfusion periods are studied [53]. Much of this cell death appears to be apoptotic [42,54,55], but it remains to be determined whether, like the apoptosis initiated by immunological mediators, it can be prevented by inhibitors of the \( \text{Ins}(1,4,5)\text{P}_3 \) pathway. The transient nature of the \( \text{Ins}(1,4,5)\text{P}_3 \) response in reperfusion might argue against its importance in responses requiring longer periods of ischaemia and reperfusion. However, there are two possible scenarios in which \( \text{Ins}(1,4,5)\text{P}_3 \) might have a central role in this process. First, it is conceivable that the transient rise in \( \text{Ins}(1,4,5)\text{P}_3 \) initiates disturbances in Ca\textsuperscript{2+} homeostasis which are self-perpetuating and cause a worsening Ca\textsuperscript{2+} overload, leading eventually to apoptotic death. Second, there have been a number of reports that reperfusion can lead to an upregulation of Fas expression both in vivo [55] and in isolated cardiomyocytes [56]. In the in vivo (blood perfused) heart, monocytes and neutrophils invade ischaemic and reperfused myocardium [57] but whether cells expressing Fas ligand (primarily T lymphocytes) also accumulate in reperfused myocardium has not yet been established. However, the increase in interleukin-6 under these conditions, makes this not unlikely [58].

Given the apparent requirement for \( \text{Ins}(1,4,5)\text{P}_3 \) in apoptosis in other cell types and its role in Fas-mediated responses in cardiomyocytes, more studies of \( \text{Ins}(1,4,5)\text{P}_3 \) involvement in cell death during myocardial ischaemia and reperfusion are warranted.

### 4. Heart failure

Heart failure can result from chronic inflammation and prolonged ischaemia as well as from sustained pressure overload. The contributing factors are not always clearly defined because hearts damaged by ischaemic insult or hypertrophied in response to pressure overload are commonly infiltrated by inflammatory cells including Fas-expressing cytotoxic T lymphocytes [59]. The failing myocardium is sensitive to the development of arrhythmias [60,61] and undergoes myocyte death both by necrotic and apoptotic mechanisms [62]. As described earlier for ischaemic and reperfusion conditions, the failing myocardium also experiences an increase in the relative activity of \( \alpha_1 \)-adrenergic receptors but the mechanisms involved are likely different and derive primarily from a marked reduction in \( \beta \)-adrenergic activity [63] resulting in an increase in the relative importance of \( \alpha_1 \)-receptor-mediated responses. The role of \( \alpha_1 \)-receptors in the initiation of cardiac hypertrophy, in the neonatal cardiomyocyte model as well as in rat heart in vivo is well established [64–66].
and recent studies have pointed to a substantial role of Gq, the G protein mediating α1-receptor activated Ins(1,4,5)P3 generation, in the development of hypertrophy and subsequent heart failure in vivo [67]. There is, as yet, no direct evidence for enhanced generation of Ins(1,4,5)P3 in failing myocardium, but indirect evidence for an alteration in Ins(1,4,5)P3 responsiveness has been presented. Transplanted hearts from patients with dilated cardiomyopathies expressed higher levels of mRNA encoding Ins(1,4,5)P3 receptors compared with well matched non-failing controls [68]. Increased Ins(1,4,5)P3 receptor content could potentially increase the sensitivity of the failing myocardium to Ins(1,4,5)P3, and thus sensitize the myocardium to arrhythmias and cell death initiated by Ins(1,4,5)P3.

5. Summary and conclusions

There is now substantial evidence that Ins(1,4,5)P3, by virtue of its role in Ca2+ release, can initiate electrophysiological disturbances which develop into cardiac arrhythmias, and in addition appears to be involved in the progression of the cardiomyocytes to apoptosis. Therapeutic regimens targeting the generation or the actions of Ins(1,4,5)P3 may thus prove beneficial in treating or preventing a number of different cardiac pathologies.

References

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