Inositol 1, 4, 5-Trisphosphate Receptors
“Exciting” Players in Cardiac Excitation-Contraction Coupling?
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Ca2+ release through ryanodine receptor (RyR) intracellular Ca2+ release channels plays the leading role in the regulation of myocyte contraction. However, Ca2+ signals arising from their less abundant inositol 1, 4, 5-trisphosphate (InsP3)-sensitive counterparts (InsP3Rs), also localized to intracellular stores, are emerging as an important modulator of Ca2+-sensitive processes in cardiac myocytes. In this week’s issue of Circulation, Signore et al report that activation of InsP3R Ca2+ release channels by G-protein–coupled receptor (GPCR) signaling regulates the electric properties of human myocardium. Their results provide new insights into how InsP3-induced Ca2+ release modulates cardiomyocyte Ca2+ handling and contractility through influencing sarcolemmal membrane potential and ionic currents.

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Ca2+ Regulation of Myocyte Contractility

An elevation in intracellular Ca2+ (known as contractile Ca2+) is a critical step in the sequence of events called excitation-contraction coupling (ECC) that couple depolarization of the plasma membrane by the propagating action potential with myocyte contraction. Ca2+ is released from sarcoplasmic reticulum Ca2+ stores via RyRs, which are activated by Ca2+ entering the cell through L-type voltage-gated Ca2+ channels (LTCCs) opened in response to membrane depolarization. The functional coupling between Ca2+ entry through LTCCs and RyRs is enabled by the proximity of the sarcosome and sarcoplasmic reticulum, which lie 10 to 15 nm apart in specialized domains called dyads (Figure). Invaginations of the sarcosome at ~1.8-mm intervals across the myocyte distribute these signaling domains throughout the cell volume, allowing a rapid and homogenous cell-wide Ca2+ elevation during an action potential (AP).2

The opinions expressed in this article are not necessarily those of the editors or of the American Heart Association.

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InsP3R: A Different Route for Modulating Contractile Ca2+ in Ventricular Myocytes

Like RyRs, InsP3Rs are large, tetrameric, intracellular Ca2+ release channels located in the sarcoplasmic reticulum/endoplasmic reticulum intracellular Ca2+ store that are activated by Ca2+.1 Unlike RyRs, InsP3Rs also require InsP3 for activation, thereby making them subject to control by extracellular ligands that engage phospholipase C–activating plasma membrane receptors, including GPCRs and receptor tyrosine kinase.1 Of the 3 mammalian InsP3R isoforms (InsP3R1, InsP3R2, and InsP3R3), InsP3R2 predominates in cardiac muscle.4 Although InsP3Rs are much less abundant than RyRs in the heart (<1:50–1:100 of RyRs),1 substantial evidence exists for InsP3Rs and InsP3-induced Ca2+ signaling in atrial myocytes.5 However, because of their even lower expression in the ventricle, the importance and contribution of InsP3Rs to Ca2+ signals during ECC have been questioned.6

Signore et al1 report that human ventricular myocytes express functional InsP3R Ca2+ release channels that are responsible for the modulation of ECC by GPCR ligands such as ATP and endothelin-1. Although a role for InsP3 in stimulating cardiac Ca2+ release was described >2 decades ago,4 Signore et al1 are the first to show that the InsP3/InsP3R signaling pathway plays an important role in regulating ECC in human ventricular myocytes. Previous work had suggested that Ca2+ release from InsP3R channels located in dyadic cleft augments RyR-mediated ECC and contractility (Figure).7 Using an elegant combination of studies in single ventricular myocytes and intact myocardium from humans and mice, Signore et al1 demonstrate a new mechanism by which InsP3 regulates ECC: InsP3R Ca2+ release signals via an unidentified mechanism to membrane ion channels and the cardiac Na+/Ca2+ exchanger (NCX), resulting in depolarized resting membrane potential and AP prolongation (the Figure). Hence, Ca2+ release from InsP3R does not contribute to contractile Ca2+ directly; rather, the enhanced Ca2+ transients and contractility induced by GPCR ligands and InsP3R signaling are the consequence of the InsP3-induced modulation of membrane ion fluxes. Moreover, RyR inhibition did not interfere with the InsP3-induced AP regulation, yet intracellular Ca2+ buffering abolished the InsP3 effects on the membrane potential. These results suggest the existence of local Ca2+ signaling microdomains of InsP3R and plasma membrane ion channels or transporters that are distinct from the dyadic domains involved in ECC (Figure), akin to local InsP3-dependent perinuclear Ca2+ signaling involved in excitation-transcription coupling.6,8 Evidence for perinuclear Ca2+ microdomains containing InsP3Rs has been identified by Ca2+ imaging,6,8 immunofluorescence,9 and electron

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InsP₃ Signaling: Punching Above Its Weight to Control Cardiomyocyte Function

In ventricular myocardium, InsP₃, Rs are expressed at exceedingly low levels relative to RyR. The Ca²⁺ current they conduct is only a quarter of that of RyRs.³ InsP₃, is generated at a very low level in cardiac myocytes relative to other tissues,⁴ thereby limiting the number of channels opened. Together with the exponential decay in Ca²⁺ concentration with distance from the source channel,⁵ the low density and Ca²⁺ flux through InsP₃, Rs would prevent a significant elevation in intracellular Ca²⁺. How can this channel then contribute so greatly to cardiomyocyte function, in both regulating ECC and promoting hypertrophic gene transcription? A likely mechanism involves localization of the InsP₃, R proximal to its signaling effectors. Analogous to P/Q channels and BKCa channels in smooth muscle,⁶ the target of InsP₃, -mediated Ca²⁺ signals is exposed to the high concentration of Ca²⁺ at the mouth of the channel. As illustrated in the Figure, InsP₃, Rs localized to the dyad within a few nanometers of RyRs could “prime” RyRs to activation by Ca²⁺ influx via LTCCs. Moreover, the association of Ca²⁺ arising from InsP₃, Rs with resident cytosolic Ca²⁺ buffers would reduce the buffering capacity of the cytosol in the vicinity of the dyad, thereby allowing Ca²⁺ subsequently released via RyRs to have a greater impact on free Ca²⁺ levels. In support of this InsP₃, R-RyR coupling model, blockade of RyRs with ryanodine reduces the amplitude of InsP₃, -stimulated elementary Ca²⁺ release events in cardiac myocytes.⁷ This mechanism has also been proposed to explain the increased spark frequency in rabbit and rat ventricular exposed to InsP₃, ¹⁰ and may underlie the positive inotropic action of GPCR/InsP₃ signaling in human myocytes. This cross-talk between InsP₃, Rs and RyRs is likely to make a greater contribution to the augmented GPCR/InsP₃ signaling observed in hypertrophic and failing hearts that exhibit significantly elevated InsP₃, expression levels.¹¹,¹² Interestingly, Signore et al do not find evidence for InsP₃, R-RyR coupling in mouse ventricular myocytes. In mice, the positive inotropic effect of GPCR agonists and InsP₃, appears to be exclusively the consequence of AP prolongation and suggests an alternative model of coupling of InsP₃, R that controls cardiac excitation (Figure). The functional results by Signore et al¹³ suggest that in murine myocardium a pool of functional InsP₃, R exists outside the dyad that couples directly to membrane ion channels or transporters membrane. Signore et al¹³ demonstrate that GPCR/InsP₃, signaling activates NCX and suggest enhanced NCX inward currents as a culprit responsible for the InsP₃, -induced AP prolongation and membrane depolarization. However, it appears unlikely that the NCX effect reported by Signore et al¹³ is solely responsible because enhanced NCX, while prolonging the AP in the short term, would eventually deplete cellular Ca²⁺ stores and result in reduced contractility. Thus, InsP₃, signaling likely modifies other membrane ion channels that either directly prolong the cardiac AP or cause a net increase in Ca²⁺ influx into the cell. Consistent with this idea, a profound effect on membrane electrophysiology was observed in mouse ventricular myocytes exposed to Fas ligand.¹⁴ Fas ligand caused an InsP₃,-dependent increase in AP duration, depolarized resting membrane potential, decreased Iᵣ, and increased arrhythmic events.¹⁵ Furthermore, there is

*Figure.* Dual control of excitation-contraction coupling (ECC) by G-protein–coupled receptor/inositol 1,4,5-trisphosphate (GPCR/InsP₃) signaling. The sarcolemma of the cardiac myocyte forming a single t-tubule and a caveolus are shown, together with the machinery underlying ECC. These membrane regions express GPCR, L-type voltage-gated Ca²⁺ channels (LTCCs), Iᵣ, Iᵣ, Na⁺/Ca²⁺ exchanger (NCX), and sodium-hydrogen exchanger (NHE). Juxtaposed to the t-tubule and caveolae membrane domains are cisternae of the sarcoplasmic reticulum (SR), forming dyadic (red box) and peripheral couplings, respectively (purple box). The distance between the sarcolemma and underlying SR in both of these membrane domains is between 10 and 15 nm, allowing rapid Ca²⁺ diffusion across the dyadic cleft. Based on the model proposed by Signore et al, in which InsP₃,-induced Ca²⁺ release (IICR) influences NCX and prolongs action potential (AP) duration (GPCR AP) independently of Ca²⁺ release via ryanodine receptors (RyRs), InsP₃ receptors (InsP₃Rs) are localized to a junctional coupling devoid of RyRs (purple box). InsP₃Rs have also been proposed to influence Ca²⁺ release via RyRs as a result of a mechanism involving their colocalization at the dyad (red box). At this location, IICR contributes minimally to the regulation of the AP (red box) but could influence NCX activity secondary to RyR activation. InsP₃ is produced downstream of GPCR stimulation of phospholipase C (PLC) and hydrolysis of PtdInsP₂ (phosphatidylinositol 4,5-bisphosphate). PLC hydrolysis of PtdInsP₂ also generates diacylglycerol (DAG), which can subsequently activate protein kinase C (PKC). PKC influences the activity of NCX, NHE, Na⁺/K⁺, and Ca²⁺ channels on the sarcolemma (gray), which may also affect the electrophysiological properties of the ventricular cardiomyocyte. For clarity, not all proteins involved in ECC are shown here.

*Figure.* InsP₃ Signaling: Punching Above Its Weight to Control Cardiomyocyte Function

In ventricular myocardium, InsP₃, Rs are expressed at exceedingly low levels relative to RyR. The Ca²⁺ current they conduct is only a quarter of that of RyRs. InsP₃ is generated at a very low level in cardiac myocytes relative to other tissues, thereby limiting the number of channels opened. Together with the exponential decay in Ca²⁺ concentration with distance from the source channel, the low density and Ca²⁺ flux through InsP₃, Rs would prevent a significant elevation in intracellular Ca²⁺. How can this channel then contribute so greatly to cardiomyocyte function, in both regulating ECC and promoting hypertrophic gene transcription? A likely mechanism involves localization of the InsP₃, R proximal to its signaling effectors. Analogous to P/Q channels and BKCa channels in smooth muscle, the target of InsP₃, mediated Ca²⁺ signals is exposed to the high concentration of Ca²⁺ at the mouth of the channel. As illustrated in the Figure, InsP₃, Rs localized to the dyad within a few nanometers of RyRs could “prime” RyRs to activation by Ca²⁺ influx via LTCCs. Moreover, the association of Ca²⁺ arising from InsP₃, Rs with resident cytosolic Ca²⁺ buffers would reduce the buffering capacity of the cytosol in the vicinity of the dyad, thereby allowing Ca²⁺ subsequently released via RyRs to have a greater impact on free Ca²⁺ levels. In support of this InsP₃, R-RyR coupling model, blockade of RyRs with ryanodine reduces the amplitude of InsP₃, -stimulated elementary Ca²⁺ release events in cardiac myocytes. This mechanism has also been proposed to explain the increased spark frequency in rabbit and rat ventricular exposed to InsP₃, and may underlie the positive inotropic action of GPCR/InsP₃, signaling in human myocytes. This cross-talk between InsP₃, Rs and RyRs is likely to make a greater contribution to the augmented GPCR/InsP₃, signaling observed in hypertrophic and failing hearts that exhibit significantly elevated InsP₃, expression levels. Interestingly, Signore et al do not find evidence for InsP₃, R-RyR coupling in mouse ventricular myocytes. In mice, the positive inotropic effect of GPCR agonists and InsP₃, appears to be exclusively the consequence of AP prolongation and suggests an alternative model of coupling of InsP₃, R that controls cardiac excitation (Figure). The functional results by Signore et al suggest that in murine myocardium a pool of functional InsP₃, R exists outside the dyad that couples directly to membrane ion channels or transporters membrane. Signore et al demonstrate that GPCR/InsP₃, signaling activates NCX and suggest enhanced NCX inward currents as a culprit responsible for the InsP₃, -induced AP prolongation and membrane depolarization. However, it appears unlikely that the NCX effect reported by Signore et al is solely responsible because enhanced NCX, while prolonging the AP in the short term, would eventually deplete cellular Ca²⁺ stores and result in reduced contractility. Thus, InsP₃, signaling likely modifies other membrane ion channels that either directly prolong the cardiac AP or cause a net increase in Ca²⁺ influx into the cell. Consistent with this idea, a profound effect on membrane electrophysiology was observed in mouse ventricular myocytes exposed to Fas ligand. Fas ligand caused an InsP₃-,dependent increase in AP duration, depolarized resting membrane potential, decreased Iᵣ, and increased arrhythmic events. Furthermore, there is
extensive cross-talk between signaling messengers downstream of GPCR other than InsP₃ that also regulates the activity of LTCCs, NCX, and the sodium-hydrogen exchanger²⁰ (Figure), which likely are also involved in the regulation of resting membrane potential and AP duration in response to InsP₃-induced Ca²⁺ release.

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References

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