

Full-length article

Role of inositol 1,4,5-trisphosphate receptors in α_1 -adrenergic receptor-induced cardiomyocyte hypertrophy¹

Da-li LUO^{2,3,5}, Jian GAO², Xiao-mei LAN³, Gang WANG⁴, Sheng WEI⁴, Rui-ping XIAO⁴, Qi-de HAN³

²Department of Pharmacology, School of Chemical Biology & Pharmaceutical Sciences, Capital University of Medical Sciences, Beijing 100069, China; ³Institute of Cardiovascular Science at Health Science Center, Peking University, Beijing 100083, China; ⁴Institute of Molecular Medicine and College of Life Science, Peking University, Beijing 100871, China

Key words

inositol 1,4,5-trisphosphate receptors; Ca^{2+} sparks; α_1 adrenergic stimulation; cardiac hypertrophy

¹ Project supported by the National Natural Science Foundation of China (No 30470692).

⁵ Correspondence to Dr Da-li LUO.

Phn 86-10-8391-1519.

Fax 86-10-8391-1520.

E-mail luodl@bjmu.edu.cn

Received 2006-04-18

Accepted 2006-05-17

doi: 10.1111/j.1745-7254.2006.00382.x

Abstract

Aim: Intracellular Ca^{2+} plays pivotal roles in diverse cellular functions, including gene transcription that underlies cardiac remodeling during stress responses. However, the role of inositol 1,4,5-trisphosphate receptors (IP_3Rs) in the mediation of cardiac intracellular Ca^{2+} and hypertrophic growth remains elusive. Prior work with neonatal rat ventricular myocytes suggests that activation of IP_3Rs may be linked to α_1 adrenergic receptor ($\alpha_1\text{AR}$) increased stereotyped Ca^{2+} spark occurrence and global Ca^{2+} oscillations. Thus, we hypothesized that Ca^{2+} release through IP_3Rs was necessary for $\alpha_1\text{AR}$ -stimulated cardiac hypertrophy. **Methods:** We used myoinositol 1,4,5-trisphosphate hexakis (butyryloxymethyl) ester (IP_3BM), a membrane-permeant ester of IP_3 , to activate IP_3Rs directly, and Fluo 4/AM to measure intracellular Ca^{2+} signaling. **Results:** IP_3BM ($10\ \mu\text{mol}\cdot\text{L}^{-1}$) mimicked the effects of phenylephrine, a selective agonist of $\alpha_1\text{AR}$, in increments in local Ca^{2+} spark release (especially in the perinuclear area) and global Ca^{2+} transient frequencies. More importantly, IP_3R inhibitors, 2-aminoethoxydiphenyl borate and Xestospongine C, abolished the IP_3BM -induced Ca^{2+} responses, and significantly suppressed $\alpha_1\text{AR}$ -induced cardiomyocyte hypertrophy assayed by cell size, [^3H] leucine incorporation and atrial natriuretic factor gene expression, during sustained (48 h) phenylephrine stimulation. **Conclusion:** These results, therefore, provide cellular mechanisms that link IP_3R signaling to $\alpha_1\text{AR}$ -stimulated gene expression and cardiomyocyte hypertrophy.

Introduction

In response to mechanical and neurohumoral stimuli, cardiac muscle undergoes adaptive hypertrophic growth, characterized by increases in myocyte size, protein synthesis and re-expression of fetal cardiac genes in order to maintain cardiac output. Although initially beneficial, sustained cardiac hypertrophy can be deleterious because of increased risk for the development of heart failure and lethal arrhythmias^[1,2].

It has been demonstrated *in vivo* and *in vitro* that, by activating phospholipase C (PLC), Gq protein-coupled receptors (GqCRs) relay the signals of mechanical overload and neurohumoral factors, such as catecholamine, angiotensin-II and endothelin-1, to initiating cellular hypertrophic

response^[3,4]. PLC catalyzes the cleavage of polyphosphoinositide into dual signaling molecules, inositol 1,4,5-trisphosphate (IP_3) and diacylglycerol (DAG), which, in turn, activate downstream protein kinases and phosphatases, such as Ca^{2+} /calmodulin (CaM)-dependent calcineurin^[5,6] and CaM kinases (CaMKs)^[7,8], and protein kinase C (PKC)^[9]. These signaling pathways then act independently or crosstalk with each other, converging on the cellular hypertrophic responses^[2,9,10]. Of these, elevation in intracellular Ca^{2+} concentration is implicated as an important factor to initiate and perpetuate GqCR-mediated cardiac hypertrophy via CaM/calcineurin- and CaMK-mediated activation of transcription factors, such as nuclear factor of activated T-cells (NFAT) and myocyte enhancer factor-2 (MEF2)^[5–9]. Understanding

the regulation of intracellular Ca^{2+} signaling by the PLC-IP₃-IP₃ receptor (IP₃R) pathway is thus crucial to understanding cellular mechanisms responsible for GqCR-mediated cardiac hypertrophy.

In the heart, the main source of Ca^{2+} in excitation-contraction is controlled by ryanodine receptors (RyRs), whereas the role of IP₃R, which express much less than RyRs, remained obscure^[11]. Recently, however, IP₃R have been found to be abundantly expressed in the embryonic heart^[12,13] and in the adult heart under conditions of heart failure and chronic arrhythmias^[14,15]. This implicates the roles of IP₃R-mediated Ca^{2+} signaling in cardiac development or remodeling in response to stress. Therefore, the present study was aimed to determine IP₃R regulation of intracellular Ca^{2+} signaling. In particular, we intended to delineate possible roles for IP₃R Ca^{2+} signaling in hypertrophic growth induced by sustained stimulation of a prototypical GqCR, α_1 adrenergic receptor (α_1 AR), in ventricular myocytes from the developing heart.

Materials and methods

Isolation and culture of myocytes Neonatal rat ventricular myocytes (NRVMs) were isolated from 1 to 2-d-old Sprague-Dawley rats by enzymatic digestion with 0.1% trypsin (Hyclone) and 0.03% collagenase (Worthington Biochemical), as described in a previous study^[16]. After incubating the cell-containing supernatant for 1.5 h to remove fibroblasts, cells were plated onto laminin-treated 35-mm dishes at a density of 1.0×10^3 – 1.2×10^3 cells/mm² unless specified otherwise. The culture medium, Dulbecco's modified Eagle's medium (DMEM) and Medium 199 (4:1) containing 10% fetal bovine serum, 4 mmol·L⁻¹ L-glutamine, 100 U/mL penicillin and streptomycin, and 0.1 mmol·L⁻¹ 5-bromo-2-deoxyuridine (Roche Molecular Biochemicals), was replaced at 42 h with serum-free medium, and cells were further cultured for 6 h. Cultured cells exhibited >95% positive staining for α -actinin, and began spontaneous contraction 24–48 h into culture.

Reverse transcription-polymerase chain reaction Myocytes were lysed with TRIzol reagent (Invitrogen) and clarified by centrifugation. Analysis of mRNA levels for atrial natriuretic factor (ANF) and 18 s were performed with primers designed to detect rat gene products. ANF detection used primers 5'-TCCCAGGCCATATTGGAGCA-3' and 5'-CAGCGAGAGCCCTCAGT-3', generating a 306 bp fragment. Detection of mRNA 18 s used primers 5'-TGCAGCCCCGGACATCTAAG-3' and 5'-GGAAGGGCACCACCAGGAGT-3', generating a 317 bp fragment. RT-PCR reactions were performed with 2 μ g of total RNA and followed by 30 cycles of

PCR amplification.

Confocal Ca^{2+} imaging NRVMs were loaded with 4 μ mol/L Fluo-4/AM (Molecular Probes) in culture medium at 37 °C for 30 min and then were washed with HEPES-buffered salt solution (mmol·L⁻¹: NaCl 135, KCl 5, MgCl₂ 1, CaCl₂ 1.8, HEPES 10 and glucose 11, with pH 7.4 adjusted by NaOH) for 20 min. Confocal images of Fluo-4 fluorescence (excitation at 488 nm and emission detection at >515 nm) were obtained using Leica SP2 inverted microscope equipped with a 63 \times oil immersion objective (NA 1.4). Time-lapsed (*xy*, 1.63 s/frame) or linescan (*xt*, 2 ms/line, 0.15 μ m/pixel) images were obtained with 1.5- μ m axial resolution. Image data analysis used customer-devised routines coded in the Interactive Data Language (IDL, Research System). All experiments were performed at room temperature (22–24 °C).

Assay of cellular hypertrophy After 6-h culture in serum-free DMEM, NRVMs at a density of 1×10^3 /mm² were stimulated with 10 μ mol·L⁻¹ phenylephrine (PE), a selective α_1 AR agonist, for 48 h to induce cell hypertrophy. In experiments with pharmacological pretreatment, designated reagents were applied 10–30 min prior to PE application and then kept in culture medium. Cell areas were measured by planimetry with 3–5 frames/dish captured at $\times 40$ magnification (Leica software) for >100 cells in each treatment. Relative rates of protein synthesis were determined by incubation of myocytes with 2 μ Ci·mL⁻¹ [³H] leucine (Amersham, UK) for 6 h and then incubated in 10% trichloroacetic acid for 30 min on ice to precipitate the protein. The precipitates were washed twice with cold water and then were solubilized in 0.1% SDS+0.3 mol·L⁻¹ NaOH at 37 °C for 1 h. [³H]leucine incorporation was then quantified for radioactivity (Beckman LS 1801) from triplicate aliquots of each sample.

Materials 2-Aminoethoxydiphenylborate (2-APB) and xestospongine C (Xe C) were from Calbiochem. Ryanodine and phenylephrine (PE) were purchased from Sigma-Aldrich. Myo-inositol 1,4,5-trisphosphate hexakis (butyryloxymethyl) ester (IP₃BM) was synthesized as described in a previous study (purity >95%)^[17].

Statistical analysis The data were analyzed and presented as mean \pm SEM. When appropriate, statistical comparison was carried out with two-way paired or unpaired Student's *t*-test. The accepted level of significance was *P*<0.05.

Results

IP₃BM-induced potentiation of Ca^{2+} oscillations In a previous study (submitted to PNAS) we identified that IP₃ formation and spontaneous global Ca^{2+} transients were increased in a dose-dependent manner after α_1 AR stimulation

with PE. Additionally, local Ca^{2+} sparks and waves, especially in the perinuclear area, were enhanced significantly upon PE stimulation, concomitant with the enriched distribution of IP_3Rs in this area. More importantly, inhibition of IP_3Rs with membrane permeable IP_3R inhibitors, 2-APB and Xe C, significantly inhibited these PE effects, suggesting an involvement of IP_3R activation for the PE-enhanced Ca^{2+} signaling in the developing cardiomyocytes.

To confirm this hypothesis, we applied IP_3BM , a membrane permeant ester of IP_3 to activate IP_3Rs directly^[17], bypassing $\alpha_1\text{AR}$. First, we used a type of non-excitabile cell, HEK293 cells, that dominantly express IP_3Rs as the intracellular Ca^{2+} release channels to identify specific properties of this synthesized compound^[18]. As reported^[17], exposure of IP_3BM ($2\text{--}25\ \mu\text{mol}\cdot\text{L}^{-1}$) for 5 min induced dose-dependent intracellular Ca^{2+} release in a Ca^{2+} -free medium, with approximately 70% of maximal Ca^{2+} release seen at a concentration of $10\ \mu\text{mol}\cdot\text{L}^{-1}$ (data not shown). Thus, IP_3BM ($10\ \mu\text{mol}\cdot\text{L}^{-1}$) stimulating NRVMs continuously for 6 min was used throughout the following study. Figure 1A shows that the frequency of spontaneous Ca^{2+} oscillations in minolayer NRVMs was increased in the presence of IP_3BM , from $5.40\pm 0.36\ \text{min}^{-1}$ in control ($n=12$) to $14.50\pm 1.38\ \text{min}^{-1}$ ($n=7$, $P<0.01$ vs control).

Pretreatment of cells with 2-APB $4\ \mu\text{mol}\cdot\text{L}^{-1}$ or Xe C $10\ \mu\text{mol}\cdot\text{L}^{-1}$ for 10 min robustly inhibited IP_3BM -induced potentiation of Ca^{2+} oscillations, whereas ryanodine, a RyRs inhibitor, at a concentration of $30\ \mu\text{mol}\cdot\text{L}^{-1}$ failed to block the IP_3BM effect (Figures 1B–D). These findings, therefore, provide direct evidence for the IP_3R signaling pathway involved in $\alpha_1\text{AR}$ potentiation of Ca^{2+} oscillations.

Subcellular responses of Ca^{2+} sparks to IP_3BM stimulation Ca^{2+} sparks are thought to constitute the elementary events of Ca^{2+} waves and global Ca^{2+} transients^[19]. Stimulation of NRVMs with PE ($10\ \mu\text{mol}\cdot\text{L}^{-1}$) could increase the spark production by 163% and 86.3% in the perinuclear and cytosol regions, respectively. To further identify the underlying molecular mechanism, we then tested this local Ca^{2+} response to IP_3BM using high-resolution linescan measurement across the nucleus of a single NRVM. We defined “perinuclear sparks” as sparks whose centers were within the nucleus and its $1\text{-}\mu\text{m}$ flanking regions seen in linescan images (Figure 2A); the rest were referred to as “cytosolic sparks”.

In IP_3BM -unstimulated myocytes, cytosolic sparks occurred at a space-time density of $1.15\pm 0.11\ (100\ \mu\text{m}\cdot\text{s})^{-1}$, and perinuclear sparks displayed a 51% higher density ($1.74\pm 0.12\ (100\ \mu\text{m}\cdot\text{s})^{-1}$, $n=32$ cells, $P<0.05$ vs cytosolic events). Similar

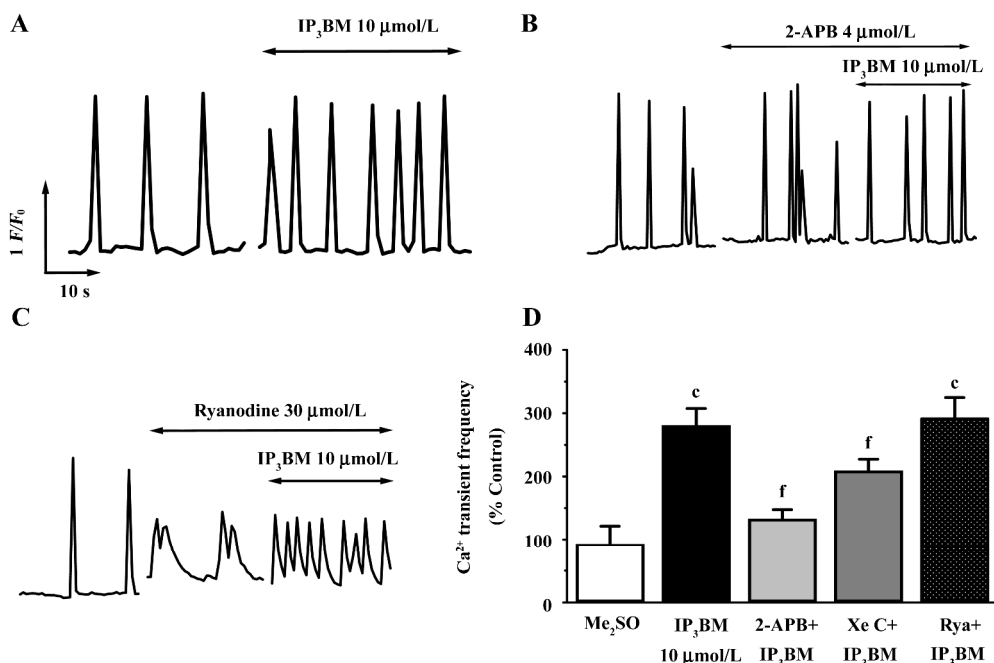


Figure 1. Effect of IP_3BM on spontaneous Ca^{2+} transients. A, Spontaneous Ca^{2+} oscillations prior to and 6 min after IP_3BM $10\ \mu\text{mol}\cdot\text{L}^{-1}$ treatment in myocytes loaded with the Ca^{2+} indicator Fluo-4. B and C, Representative effects of 2-APB (B) and ryanodine (Rya, C) on basal and IP_3BM -induced global Ca^{2+} oscillations. D, Effects of pretreatment with 2-APB, Rya and Xe C (at 4, 30 and $10\ \mu\text{mol}\cdot\text{L}^{-1}$ for 10, 10 and 30 min, respectively) on IP_3BM -induced spontaneous Ca^{2+} transients. Data are presented as percentage of vehicle control (Me_2SO , 0.1%). $n=6\text{--}8$ experiments. Mean \pm SEM. ^c $P<0.01$ vs Me_2SO . ^f $P<0.01$ vs IP_3BM group.

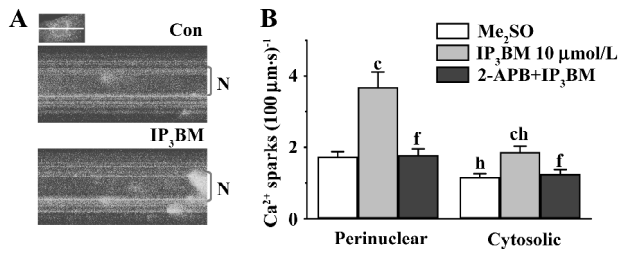


Figure 2. Modulation of perinuclear and cytosolic Ca²⁺ sparks by IP₃BM. A, Typical linescan images of Ca²⁺ sparks under control conditions (con) and in the presence of IP₃BM (10 μmol·L⁻¹, 6 min). Inset: Confocal scan line across the nucleus and the cytosol. Intense staining of Fluo-4 allowed for the identification of the nuclear membrane. The nuclear region in the linescan image is marked "N". B, Statistics of spark density [events (100 μm·s)⁻¹] for control, IP₃BM and 2-APB (pretreated cells for 10 min)+IP₃BM groups as indicated. *n*=26–32 cells. Mean±SEM. ^c*P*<0.01 vs control (Me₂SO). ^f*P*<0.01 vs IP₃BM. ^h*P*<0.05 vs perinuclear region.

to PE, IP₃BM increased spark frequency by 112% and 62% in the perinuclear and cytosol regions, respectively, rendering 1.8-fold higher perinuclear than cytosol spark density (Figure 2A, 2B), while the amplitude, width and duration of Ca²⁺ sparks were not significantly altered in either area. Concomitantly, the IP₃R inhibitor, 2-APB also completely reversed the IP₃BM-induced spark responses in both subcellular areas (Figure 2B). Therefore, these results further confirm that α₁AR stimulation profoundly modulates the genesis of Ca²⁺ sparks through IP₃R-dependent mechanism, and the spark response is greater in the perinuclear region of NRVM.

IP₃R inhibition suppressed PE-induced hypertrophic responses Finally, we sought to test the involvement of the IP₃R signaling pathway in α₁AR stimulated NRVM growth, a well-established model for cardiomyocyte hypertrophy^{12,61}. As previously reported⁶¹, NRVMs with sustained (48 h) PE stimulation manifested increased cellular size (Figure 3A, 3B) and overall protein synthesis assayed by [³H]leucine incor-

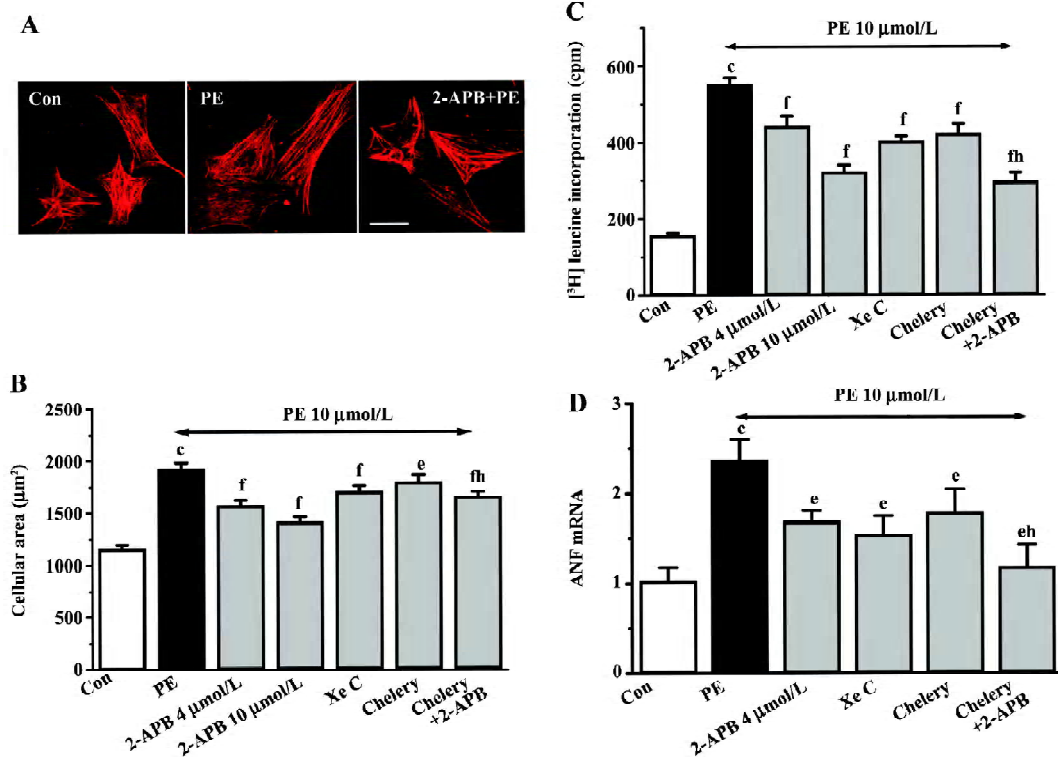


Figure 3. Inhibition of IP₃Rs suppressed PE-induced hypertrophic growth in NRVM. After being cultured for 6 h in serum-free DMEM, myocytes were incubated with 2-APB for 10 min, or chelerythrine (Chelery, PKC inhibitor), or Chelery (3 μmol·L⁻¹)+2-APB (4 μmol·L⁻¹) for 20 min, or Xe C for 30 min, respectively, followed by stimulation with 10 μmol·L⁻¹ PE for 48 h. A and B, Cellular size as delineated by staining of actin filaments (A) and analyzed using Image software (B). C, Protein synthesis was assessed by measurement of [³H] leucine uptake (see Methods) under various experimental conditions. D, Summarized data corresponding to ANF mRNA in different groups determined by RT-PCR and normalized to 18 s mRNA. *n*=4 separated experiments. Mean±SEM. ^c*P*<0.01 vs unstimulated cells (con). ^e*P*<0.05, ^f*P*<0.01 vs PE group. ^h*P*<0.05 vs 2-APB or Chelery group.

poration (Figure 3C). Additionally, mRNA expression of fetal cardiac genes such as ANF was also elevated upon PE stimulation (Figure 3D), a hallmark of cardiac hypertrophy^[20]. Inhibition of IP₃Rs with 2-APB or Xe C, at concentrations that effectively suppressed the aforementioned Ca²⁺ responses, caused a substantial suppression of PE-induced increases in cellular size, protein synthesis, and ANF mRNA level (Figure 3). Parallel experiments confirmed that PKC inhibitor, chelerythrine, also partially reversed PE-induced hypertrophic responses (Figure 3)^[9], suggesting that neither PKC nor IP₃R pathway is the exclusive mediator of the α_1 AR hypertrophic effects. Indeed, combined IP₃R and PKC inhibition appeared to exert greater effects in suppressing PE-stimulated protein synthesis (Figure 3C) and ANF expression (Figure 3D). Thus, the present and previous findings support the notion that both IP₃R and PKC pathways contribute to α_1 AR-mediated hypertrophic effects^[6,9].

To obtain more direct evidence for the role of IP₃R in initiating cardiac hypertrophic growth, we also tried IP₃BM (10 $\mu\text{mol}\cdot\text{L}^{-1}$) in parallel experiments, but we failed to induce significant cardiomyocyte hypertrophic growth by this compound, this is likely because of its quick metabolism of IP₃ inside the cells.

Discussion

The present study provides direct evidence for IP₃R activation as a cellular mechanism underlying α_1 AR stimulated Ca²⁺ signaling and, more importantly, linking to hypertrophic growth in ventricular myocytes from the developing rat heart. Hierarchical Ca²⁺ events in NRVMs, ranging from local Ca²⁺ sparks, waves to global Ca²⁺ transients, manifest a rather complex architecture of intracellular Ca²⁺ signaling. Unlike IP₃, IP₃BM can enter into cells and release free IP₃ inside, enabling us to observe Ca²⁺ signaling response to IP₃R activation in intact cells. At the cellular level, IP₃BM stimulation increases the frequency of spontaneous Ca²⁺ transients to 3-fold (Figure 1). At the elementary release level, the action of IP₃BM is characterized by an increase in the rate of spark occurrence, with unitary properties remaining stereotypical. The spark response is spatially uneven: the perinuclear sarcoplasmic reticulum manifests a greater increase in spark production (Figure 2), concomitant with the subcellular distribution of IP₃Rs in NRVMs (data not shown here). These similar responses in local as well as global Ca²⁺ signaling to IP₃BM and α_1 AR stimulation are all sensitive to inhibition of IP₃Rs, further confirming activation of IP₃Rs as the key mechanism in α_1 AR mediated Ca²⁺ signaling.

Intracellular Ca²⁺, particularly nuclear Ca²⁺, plays an important role in the regulation of gene expression that under-

lies cardiac remodeling during stress responses^[1,4,5,21]. Accumulating evidence has indicated that the enhancement of Ca²⁺ signaling activates multiple downstream signaling factors that induce cardiac hypertrophic growth. For instance, an increase of Ca²⁺ in cardiomyocytes activates Ca²⁺-dependent phosphatase calcineurin, resulting in dephosphorylates of transcription factors known as NFAT3, which, in turn, binds upon dephosphorylation to the transcription factor GATA4 to upregulate cardiac-specific gene expression^[5,6,22]. However, Ca²⁺ signaling also activates CaMK, which then stimulate several transcription factors, including MEF2, and initiates hypertrophic gene expressions^[7,8]. More recently, IP₃Rs have been found to be confined to the nuclear envelope of adult rat ventricular myocytes, and associated with the activation of local CaMK II^[23], implying GqCR-IP₃R-Ca²⁺-CaMK II may converge to a signaling pathway at the site of the nucleus.

The observation that PE (data not shown) and IP₃BM similarly and profoundly alter Ca²⁺ signaling, including sparks, waves and Ca²⁺ transient frequency, and the fact that these alterations occur preferentially in the perinuclear region implicate a role for IP₃Rs in α_1 AR-stimulated NRVM growth. While previous studies have linked the hypertrophic responses to the DAG-PKC branch of the α_1 AR signaling cascades^[9], a major finding of the present work is that IP₃R inhibitors, at concentrations that effectively block α_1 AR- and IP₃BM-induced Ca²⁺ responses, significantly attenuate ANF expression, protein synthesis and cell growth induced by sustained (48 h) PE stimulation. Furthermore, we demonstrated that the hypertrophic effect of α_1 AR stimulation depended on the concurrent IP₃-IP₃R pathway and DAG-PKC pathway (Figure 3). Either inhibition partially reverses the hypertrophic growth while inhibition of dual pathways exerts greater inhibitory effects, thus reconciling our new finding with those in the published literature^[24,25].

In summary, we have demonstrated that IP₃R activation plays an important role in triggering local as well as global Ca²⁺ release in α_1 AR stimulated NRVM, and the altered intracellular Ca²⁺ signaling is in part responsible for catecholamine-induced cardiomyocyte hypertrophic growth.

References

- 1 Sugden PH, Clerk A. Cellular mechanisms of cardiac hypertrophy. *J Mol Med* 1998; 76: 725–46.
- 2 Yamamoto K, Dang QN, Maeda Y, Huang H, Kelly RA, Lee RT. Regulation of cardiomyocyte mechanotransduction by the cardiac cycle. *Circulation* 2001; 103: 1459–64.
- 3 Wetschurck N, Rütten H, Zywiets A, Gehring D, Wilkie TM, Chen J, *et al*. Absence of pressure overload induced myocardial hypertrophy after conditional inactivation of G α_q /G α_{11} in

- cardiomyocytes. *Nat Med* 2001; 7: 1236–40.
- 4 Sabri A, Wilson BA, Steinberg SF. Dual actions of the $G\alpha_q$ agonist *pasteurella multocida* toxin to promote cardiomyocyte hypertrophy and enhance apoptosis susceptibility. *Circ Res* 2002; 90: 850–7.
 - 5 Crabtree GR. Generic signals and specific outcomes: signaling through Ca^{2+} , calcineurin, and NF-AT. *Cell* 1999; 96: 611–4.
 - 6 Taigen T, De Windt LJ, Lim HW, Molkenin JD. Targeted inhibition of calcineurin prevents agonist-induced cardiomyocyte hypertrophy. *Proc Natl Acad Sci USA* 2000; 97: 1196–201.
 - 7 Blaeser F, Ho N, Prywes R, Chatila TA. Ca^{2+} -dependent gene expression mediated by MEF2 transcription factors. *J Biol Chem* 2000; 275: 197–209.
 - 8 Akazawa H, Komuro I. Roles of cardiac transcription factors in cardiac hypertrophy. *Circ Res* 2003; 92: 1079–88.
 - 9 Muth JN, Bodi I, Lewis W, Varadi G, Schwartz A. A Ca^{2+} -dependent transgenic model of cardiac hypertrophy: a role for protein kinase Calpha. *Circulation* 2001; 103: 140–7.
 - 10 De Windt LJ, Lim HW, Haq S, Force T, Molkenin JD. Calcineurin promotes protein kinase C and c-Jun NH_2 -terminal kinase activation in the heart. Cross-talk between cardiac hypertrophic signaling pathways. *J Biol Chem* 2000; 275: 13571–9.
 - 11 Marks AR. Cardiac intracellular calcium release channels—role in heart failure. *Circ Res* 2000; 87: 8–11.
 - 12 Rosemblyt N, Moschella MC, Ondriasa E, Gutstein DE, Ondrias K, Marks AR. Intracellular calcium release channel expression during embryogenesis. *Dev Biol* 1999; 206: 163–77.
 - 13 Kolossov E, Fleischmann BK, Liu Q, Bloch W, Viatchenko-Karpinski S, Manzke O, *et al*, Functional characteristics of ES cell-derived cardiac precursor cells identified by tissue-specific expression of the green fluorescent protein. *J Cell Biol* 1998; 143: 2045–56.
 - 14 Go LO, Moschella MC, Watras J, Handa KK, Fyfe BS, Marks AR. Differential regulation of two types of intracellular calcium release channels during end-stage heart failure. *J Clin Invest* 1995; 95: 888–94.
 - 15 Ai X, Curran JW, Shannon TR, Bers DM, Pogwizd SM. Ca^{2+} /calmodulin-dependent protein kinase modulates cardiac ryanodine receptor phosphorylation and sarcoplasmic reticulum Ca^{2+} leak in heart failure. *Circ Res* 2005; 97: 1314–22.
 - 16 Chesley A, Lundberg MS, Asai T, Xiao RP, Ohtani S, Lakatta EG, *et al*. The beta(2)-adrenergic receptor delivers an antiapoptotic signal to cardiac myocytes through G(i)-dependent coupling to phosphatidylinositol 3'-kinase. *Circ Res* 2000; 87: 1172–9.
 - 17 Li W, Schultz C, Llopis J, Tsien RY. Membrane-permeant esters of inositol polyphosphates, chemical syntheses and biological applications. *Tetrahedron* 1997; 53: 12017–40.
 - 18 Venkatachalam K, van Rossum DB, Patterson RL, Ma HT, Gill DL. The cellular and molecular basis of store-operated calcium entry. *Nat Rev Mol Cell Biol* 2002; 4: 263–72.
 - 19 Cheng H, Lederer WJ, Cannel MB. Calcium sparks: the elementary events underlying excitation-contraction coupling in heart muscle. *Science* 1993; 262: 740–4.
 - 20 Wang Y, Huang S, Sah VP, Ross J Jr, Brown JH, Han J, *et al*. Cardiac muscle cell hypertrophy and apoptosis induced by distinct members of the p38 mitogen-activated protein kinase family. *J Biol Chem* 1998; 273: 2161–8.
 - 21 Frey N, McKinsey TA, Olson EN. Decoding calcium signals involved in cardiac growth and function. *Nat Med* 2000; 6: 1221–7.
 - 22 Molkenin JD, Lu JR, Antos CL, Markham B, Richardson J, Robbins J, *et al*. A calcineurin-dependent transcriptional pathway for cardiac hypertrophy. *Cell* 1998; 93: 215–28.
 - 23 Bare DJ, Kettlun CS, Liang M, Ber DM, Mignery GA. Cardiac type 2 inositol 1,4,5-trisphosphate receptor: interaction and modulation by calcium/calmodulin-dependent protein kinase II. *J Biol Chem* 2005; 280: 15912–20.
 - 24 Wu GY, Toyokawa T, Hahn H, Dorn GW. Epsilon protein kinase C in pathological myocardial hypertrophy. Analysis by combined transgenic expression of translocation modifiers and Galphaq. *J Biol Chem* 2000; 275: 29927–30.
 - 25 Pan J, Fukuda K, Saito M, Matsuzaki J, Kodama H, Sano M, *et al*. Mechanical stretch activates the JAK/STAT pathway in rat cardiomyocytes. *Circ Res* 1999; 84: 1127–36.