Blackwell
Publishing

Full-length article

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

Da-li LUO[2,3,5], Jian GAO[2], Xiao-mei LAN[3], Gang WANG[4], Sheng WEI[4], Rui-ping XIAO[4], Qi-de HAN[3]

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

Key words

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$_3$Rs) 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$_3$Rs may be linked to α_1 adrenergic receptor (α_1AR) increased stereotyped Ca^{2+} spark occurrence and global Ca^{2+} oscillations. Thus, we hypothesized that Ca^{2+} release through IP$_3$Rs was necessary for α_1AR-stimulated cardiac hypertrophy. **Methods:** We used myoinositol 1,4,5-trisphosphate hexakis (butyryloxymethyl) ester (IP$_3$BM), a membrane-permeant ester of IP$_3$, to activate IP$_3$Rs directly, and Fluo 4/AM to measure intracellular Ca^{2+} signaling. **Results:** IP$_3$BM (10 μmol·L^{-1}) mimicked the effects of phenylephrine, a selective agonist of α_1AR, in increments in local Ca^{2+} spark release (especially in the perinuclear area) and global Ca^{2+} transient frequencies. More importantly, IP$_3$R inhibitors, 2-aminoethoxydiphenyl borate and Xestospongin C, abolished the IP$_3$BM-induced Ca^{2+} responses, and significantly suppressed α_1AR-induced cardiomyocyte hypertrophy assayed by cell size, [^{3}H] leucine incorporation and atrial natriuretic factor gene expression, during sustained (48 h) phenylephrine stimulation. **Conclusion:** These results, therefore, provide cellular mechanisms that link IP$_3$R signaling to α_1AR-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/carcineurin- 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-IP$_3$-IP$_3$ receptor (IP$_3$R) 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 IP$_3$Rs, which express much less than RyRs, remained obscure[11]. Recently, however, IP$_3$Rs 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 IP$_3$R-mediated Ca^{2+} signaling in cardiac development or remodeling in response to stress. Therefore, the present study was aimed to determine IP$_3$R regulation of intracellular Ca^{2+} signaling. In particular, we intended to delineate possible roles for IP$_3$R Ca^{2+} signaling in hypertrophic growth induced by sustained stimulation of a prototypical GqCR, α_1 adrenergic receptor (α_1AR), 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/mm^2 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'-TGCAGCCCCGGA-CATCTAAG-3' and 5'-GGAAGGGCACCACCAGGAGT-3', generating a 317 bp fragment. RT-PCR reactions were performed with 2 μg of total RNA and followed by 30 cycles of PCR amplification.

Confocal Ca^{2+} imaging NRVMs were loaded with 4 μmol/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, MgCl$_2$ 1, CaCl$_2$ 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×oil immersion objective (NA 1.4). Time-lapsed (xy, 1.63 s/frame) or linescan (xt, 2 ms/line, 0.15 μm/pixel) images were obtained with 1.5-μm 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/mm^2 were stimulated with 10 μmol·L^{-1} phenylephrine (PE), a selective α_1AR 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 ×40 magnification (Leica software) for >100 cells in each treatment. Relative rates of protein synthesis were determined by incubation of myocytes with 2 μCi·mL^{-1} [^{3}H] 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. [^{3}H]leucine incorporation was then quantified for radioactivity (Beckman LS 1801) from triplicate aliquots of each sample.

Materials 2-Aminoethoxydiphenylborate (2-APB) and xestospongin C (Xe C) were from Calbiochem. Ryanodine and phenylephrine (PE) were purchased from Sigma-Aldrich. Myo-inositol 1,4,5-trisphosphate hexakis (butyryloxymethyl) ester (IP$_3$BM) was synthesized as described in a previous study (purity >95%)[17].

Statistical analysis The data were analyzed and presented as mean±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

IP$_3$BM-induced potentiation of Ca^{2+} oscillations In a previous study (submitted to PNAS) we identified that IP$_3$ formation and spontaneous global Ca^{2+} transients were increased in a dose-dependent manner after α_1AR 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 α_1AR. First, we used a type of non-excitable 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 (2–25 $\mu mol\cdot L^{-1}$) 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 $\mu mol\cdot L^{-1}$ (data not shown). Thus, IP_3BM (10 $\mu mol\cdot L^{-1}$) 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±0.36 min^{-1} in control (n=12) to 14.50±1.38 min^{-1} (n=7, $P<0.01$ *vs* control).

Pretreatment of cells with 2-APB 4 $\mu mol\cdot L^{-1}$ or Xe C 10 $\mu mol\cdot L^{-1}$ for 10 min robustly inhibited IP_3BM-induced potentiation of Ca^{2+} oscillations, whereas ryanodine, a RyRs inhibitor, at a concentration of 30 $\mu mol\cdot L^{-1}$ failed to block the IP_3BM effect (Figures 1B–D). These findings, therefore, provide direct evidence for the IP_3R signaling pathway involved in α_1AR 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 $\mu mol\cdot L^{-1}$) 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-μm 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±0.11 (100 $\mu m\cdot s)^{-1}$, and perinuclear sparks displayed a 51% higher density (1.74±0.12 (100 $\mu m\cdot s)^{-1}$, n=32 cells, $P<0.05$ *vs* cytosolic events). Similar
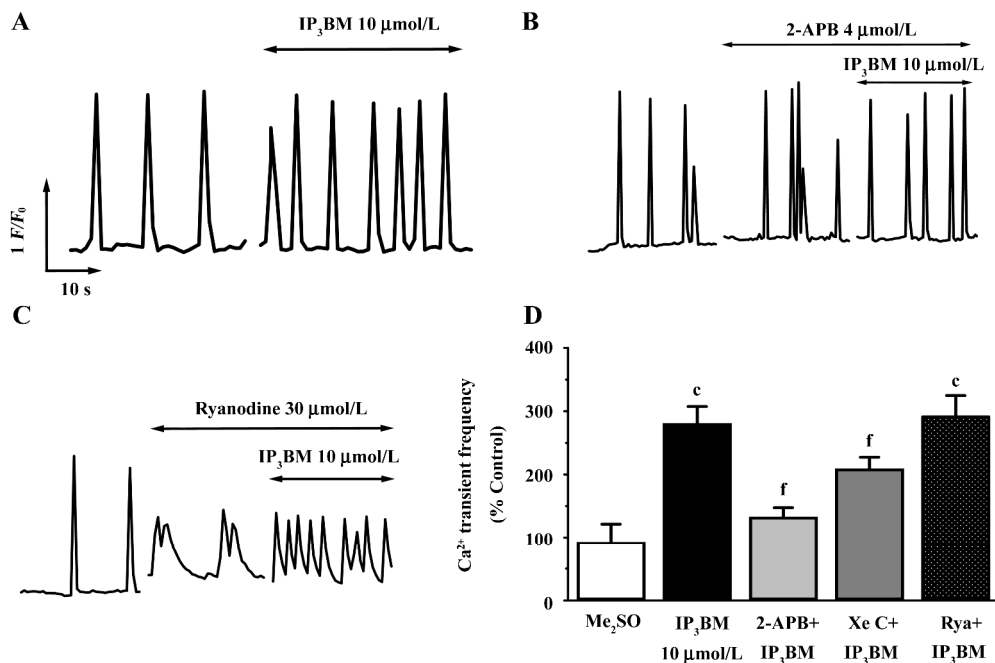

Figure 1. Effect of IP_3BM on spontaneous Ca^{2+} transients. A, Spontaneous Ca^{2+} oscillations prior to and 6 min after IP_3BM 10 $\mu mol\cdot L^{-1}$ treatment in myocytes loaded with the Ca^{2+} indicator Fluo-4. B and C, Representative effects of 2-APB (B) and ryanodine (Rya, C) on basal and IP_3BM-induced global Ca^{2+} oscillations. D, Effects of pretreatment with 2-APB, Rya and Xe C (at 4, 30 and 10 $\mu mol\cdot L^{-1}$ 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=6−8 experiments. Mean±SEM. $^cP<0.01$ *vs* Me_2SO. $^fP<0.01$ *vs* IP_3BM group.
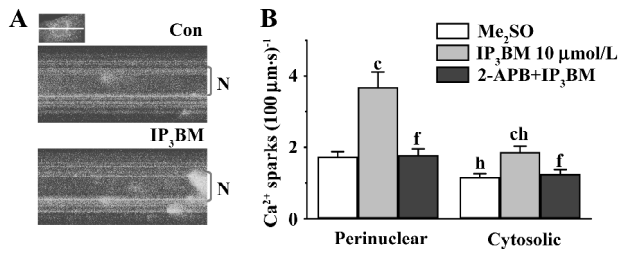
Figure 2. Modulation of perinuclear and cytosolic Ca^{2+} sparks by IP_3BM. A, Typical linescan images of Ca^{2+} sparks under control conditions (con) and in the presence of IP_3BM (10 $\mu mol \cdot L^{-1}$, 6 min). Inset: Confocal scan line across the nucleus and the cytosol. Intense staining of Fluo-4 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 $\mu m \cdot s)^{-1}$] for control, IP_3BM and 2-APB (pretreated cells for 10 min)+IP_3BM groups as indicated. n=26–32 cells. Mean±SEM. [c]$P<0.01$ *vs* control (Me$_2$SO). [f]$P<0.01$ *vs* IP_3BM. [h]$P<0.05$ *vs* perinuclear region.

to PE, IP_3BM 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 Ca^{2+} sparks were not significantly altered in either area. Concomitantly, the IP_3R inhibitor, 2-APB also completely reversed the IP_3BM-induced spark responses in both subcellular areas (Figure 2B). Therefore, these results further confirm that α_1AR stimulation profoundly modulates the genesis of Ca^{2+} sparks through IP_3R-dependent mechanism, and the spark response is greater in the perinuclear region of NRVM.

IP_3R inhibition suppressed PE-induced hypertrophic responses Finally, we sought to test the involvement of the IP_3R signaling pathway in α_1AR stimulated NRVM growth, a well-established model for cardiomyocyte hypertrophy[2,6]. As previously reported[6], NRVMs with sustained (48 h) PE stimulation manifested increased cellular size (Figure 3A, 3B) and overall protein synthesis assayed by [^{3}H]leucine incor-
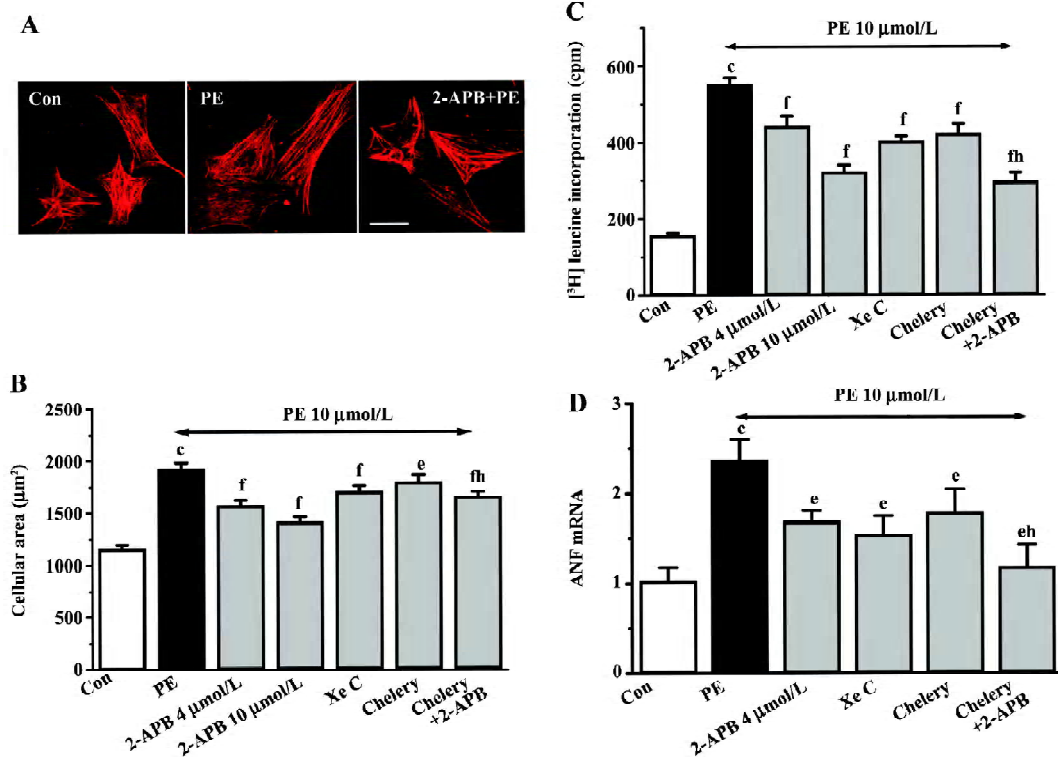

Figure 3. Inhibition of IP_3Rs suppressed PE-induced hypertrophic growth in NRVM. After being cultured for 6 h in serum-free DMEM, myocytes were incubated with 2-APB for 10 min, or chelerythrine (Chelery, PKC inhibitor), or Chelery (3 $\mu mol \cdot L^{-1}$)+2-APB (4 $\mu mol \cdot L^{-1}$) for 20 min, or Xe C for 30 min, respectively, followed by stimulation with 10 $\mu mol \cdot L^{-1}$ 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 [^{3}H] 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 IP$_3$Rs with 2-APB or Xe C, at concentrations that effectively suppressed the aforementioned Ca^{2+} 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 IP$_3$R pathway is the exclusive mediator of the α$_1$AR hypertrophic effects. Indeed, combined IP$_3$R 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 IP$_3$R and PKC pathways contribute to α$_1$AR-mediated hypertrophic effects[6,9].

To obtain more direct evidence for the role of IP$_3$R in initiating cardiac hypertrophic growth, we also tried IP$_3$BM (10 μmol·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 IP$_3$ inside the cells.

Discussion

The present study provides direct evidence for IP$_3$R activation as a cellular mechanism underlying α$_1$AR stimulated Ca^{2+} signaling and, more importantly, linking to hypertrophic growth in ventricular myocytes from the developing rat heart. Hierarchical Ca^{2+} events in NRVMs, ranging from local Ca^{2+} sparks, waves to global Ca^{2+} transients, manifest a rather complex architecture of intracellular Ca^{2+} signaling. Unlike IP$_3$, IP$_3$BM can enter into cells and release free IP$_3$ inside, enabling us to observe Ca^{2+} signaling response to IP$_3$R activation in intact cells. At the cellular level, IP$_3$BM stimulation increases the frequency of spontaneous Ca^{2+} transients to 3-fold (Figure 1). At the elementary release level, the action of IP$_3$BM 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 IP$_3$Rs in NRVMs (data not shown here). These similar responses in local as well as global Ca^{2+} signaling to IP$_3$BM and α$_1$AR stimulation are all sensitive to inhibition of IP$_3$Rs, further confirming activation of IP$_3$Rs as the key mechanism in α$_1$AR mediated Ca^{2+} signaling.

Intracellular Ca^{2+}, particularly nuclear Ca^{2+}, plays an important role in the regulation of gene expression that underlies cardiac remodeling during stress responses[1,4,5,21]. Accumulating evidence has indicated that the enhancement of Ca^{2+} signaling activates multiple downstream signaling factors that induce cardiac hypertrophic growth. For instance, an increase of Ca^{2+} in cardiomyocytes activates Ca^{2+}-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, Ca^{2+} signaling also activates CaMK, which then stimulate several transcription factors, including MEF2, and initiates hypertrophic gene expressions[7,8]. More recently, IP$_3$Rs 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-IP$_3$R-Ca^{2+}-CaMK II may converge to a signaling pathway at the site of the nucleus.

The observation that PE (data not shown) and IP$_3$BM similarly and profoundly alter Ca^{2+} signaling, including sparks, waves and Ca^{2+} transient frequency, and the fact that these alterations occur preferentially in the perinuclear region implicate a role for IP$_3$Rs 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 IP$_3$R inhibitors, at concentrations that effectively block α$_1$AR- and IP$_3$BM-induced Ca^{2+} 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 IP$_3$-IP$_3$R 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 IP$_3$R activation plays an important role in triggering local as well as global Ca^{2+} release in α$_1$AR stimulated NRVM, and the altered intracellular Ca^{2+} 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   Wettschureck N, Rütten H, Zywietz A, Gehring D, Wilkie TM, Chen J, *et al*. Absence of pressure overload induced myocardial hypertrophy after conditional inactivation of Gα$_q$/Gα$_{11}$ in

cardiomyocytes. Nat Med 2001; 7: 1236–40.

4   Sabri A, Wilson BA, Steinberg SF. Dual actions of the Gα_q ago-
    nist *pasteurella multocida* toxin to promote cardiomyocyte hy-
    pertrophy 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, Molkentin 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+}-depen-
    dent 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, Molkentin JD. Calcineurin
    promotes protein kinase C and c-Jun NH_2-terminal kinase acti-
    vation 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 Rosemblit 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 re-
    lease channels during end-stage heart failure. J Clin Invest 1995;
    95: 888–94.

15 Ai X, Curran JW, Shannon TR, Bers DM, Pogwizd SM. $Ca^{2+}/$

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 elemen-
    tary 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 dis-
    tinct 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 Molkentin JD, Lu JR, Antos CL, Markham B, Richardson J,
    Robbins J, *et al*. A calcineurin-dependent transcriptional path-
    way 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.

calmodulin-dependent protein kinase modulates cardiac ryanodine
receptor phosphorylation and sarcoplasmic reticulum Ca^{2+} leak
in heart failure. Circ Res 2005; 97: 1314–22.