Acta Pharmacologica Sinica 2006 Jul; 27 (7): 895–900

Blackwell Publishing

Full-length article

# Role of inositol 1,4,5-trisphosphate receptors in $\alpha_1$-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

inositol 1,4,5-trisphosphate receptors; $Ca^{2+}$ sparks; $\alpha_1$ 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 $\alpha_1$ adrenergic receptor ($\alpha_1$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 $\alpha_1$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 $\mu mol \cdot L^{-1}$) mimicked the effects of phenylephrine, a selective agonist of $\alpha_1$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 Xestospongin C, abolished the $IP_3BM$-induced $Ca^{2+}$ responses, and significantly suppressed $\alpha_1$AR-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 $IP_3R$ signaling to $\alpha_1$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/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 ($IP_3R$) 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 $IP_3Rs$, which express much less than RyRs, remained obscure[11]. Recently, however, $IP_3Rs$ 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 $IP_3R$-mediated $Ca^{2+}$ signaling in cardiac development or remodeling in response to stress. Therefore, the present study was aimed to determine $IP_3R$ regulation of intracellular $Ca^{2+}$ signaling. In particular, we intended to delineate possible roles for $IP_3R$ $Ca^{2+}$ signaling in hypertrophic growth induced by sustained stimulation of a prototypical GqCR, $\alpha_1$ adrenergic receptor ($\alpha_1$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 $1.0\times10^3$–$1.2\times10^3$ 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** Myoc ytes 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 fragm ent. 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 μg of total RNA and followed by 30 cycles of PCRamplification.

**Confocal $Ca^{2+}$ imaging** NRVMs were loaded with 4 μmol/L Fluo-4/AM (Molecular Probes) in culture medium at 37 ºC 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 ºC).

**Assay of cellular hypertrophy** After 6-h culture in serum-free DMEM, NRVMs at a density of $1\times10^3$/mm$^2$ were stimulated with 10 μmol·L$^{-1}$ phenylephrine (PE), a selective $\alpha_1$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 ×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 ºC 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 ($IP_3BM$) 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

**$IP_3BM$-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 $\alpha_1$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 $\alpha_1AR$. 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 μmol·$L^{-1}$) 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 μmol·$L^{-1}$ (data not shown). Thus, $IP_3BM$ (10 μmol·$L^{-1}$) 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 μmol·$L^{-1}$ or Xe C 10 μmol·$L^{-1}$ for 10 min robustly inhibited $IP_3BM$-induced potentiation of $Ca^{2+}$ oscillations, whereas ryanodine, a RyRs inhibitor, at a concentration of 30 μmol·$L^{-1}$ failed to block the $IP_3BM$ effect (Figures 1B–D). These findings, therefore, provide direct evidence for the $IP_3R$ signaling pathway involved in $\alpha_1AR$ 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 μmol·$L^{-1}$) 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 μm·s)$^{-1}$, and perinuclear sparks displayed a 51% higher density (1.74±0.12 (100 μm·s)$^{-1}$, $n$=32 cells, $P$<0.05 *vs* cytosolic events). Similar

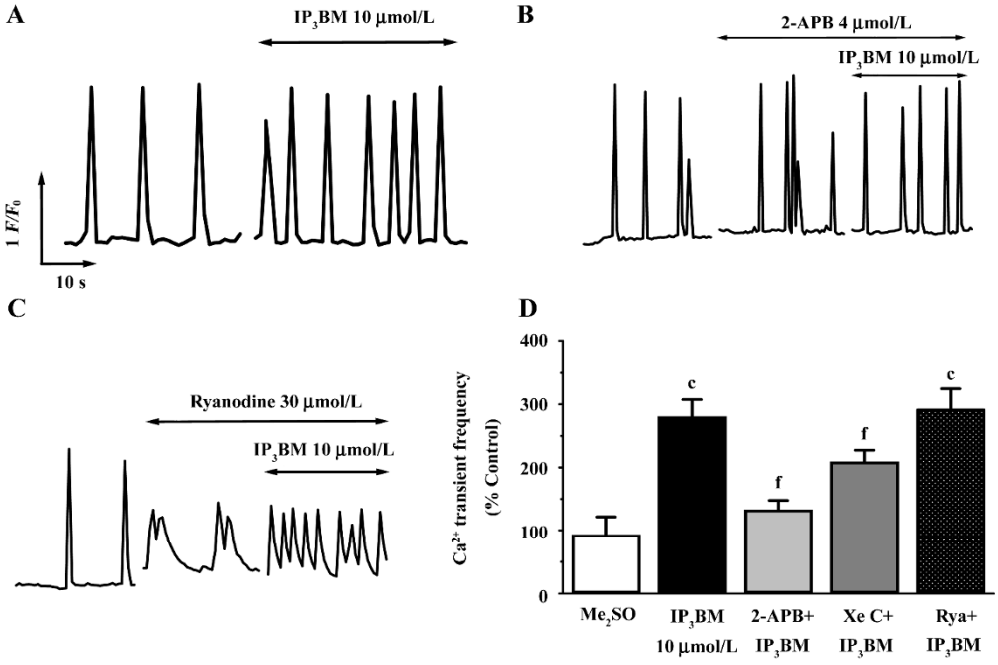


**Figure 1.** Effect of $IP_3BM$ on spontaneous $Ca^{2+}$ transients. A, Spontaneous $Ca^{2+}$ oscillations prior to and 6 min after $IP_3BM$ 10 μmol·$L^{-1}$ treatment in myocytes loaded with the $Ca^{2+}$ indicator Fluo-4. B and C, Representative effects of 2-APB (B) and ryanodine (Rya, C) on basal and $IP_3BM$-induced global $Ca^{2+}$ oscillations. D, Effects of pretreatment with 2-APB, Rya and Xe C (at 4, 30 and 10 μmol·$L^{-1}$ 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.

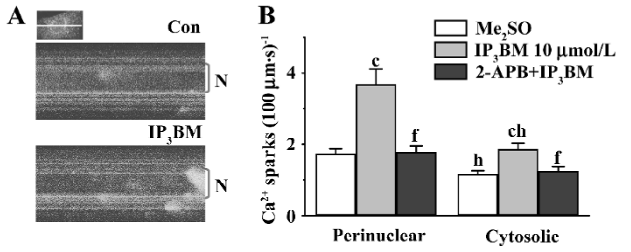


**Figure 2.** Modulation of perinuclear and cytosolic $Ca^{2+}$ sparks by $IP_3BM$. A, Typical linescan images of $Ca^{2+}$ sparks under control conditions (con) and in the presence of $IP_3BM$ (10 μmol·$L^{-1}$, 6 min). Inset: Confocal scan line across the nucleus and the cytosol. Intense staining of 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, $IP_3BM$ 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 ($Me_2SO$). $^{f}P$<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 $\alpha_1$AR 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 $\alpha_1$AR 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-

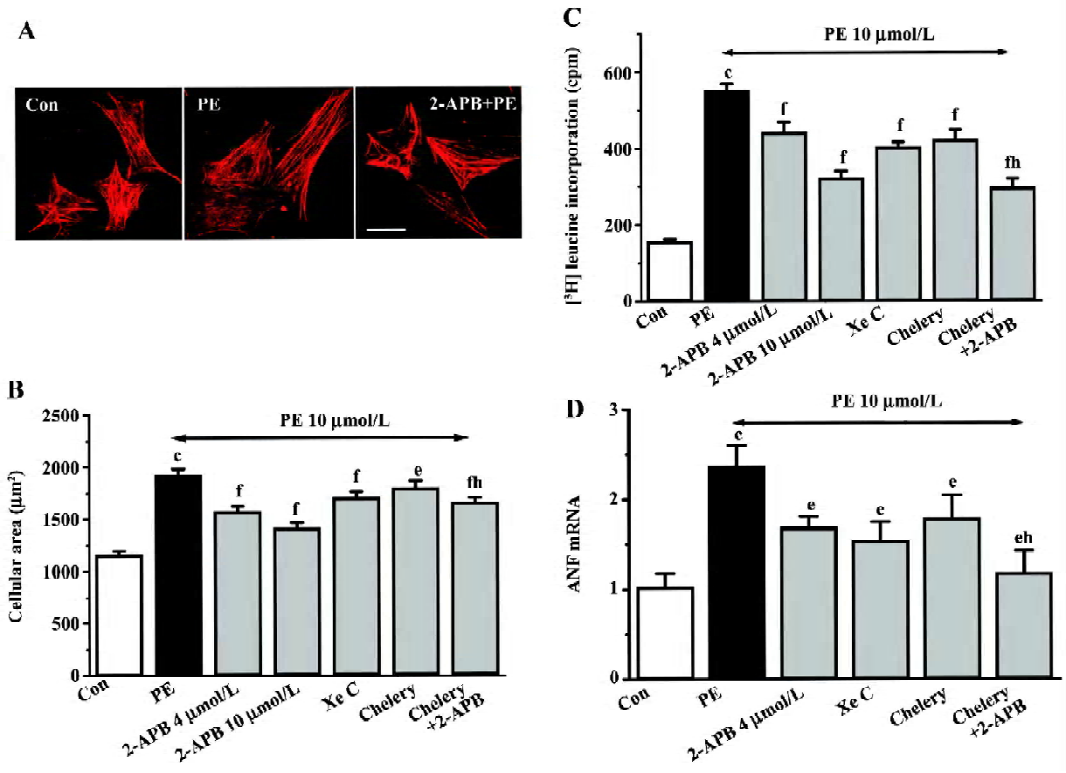


**Figure 3.** Inhibition of $IP_3Rs$ suppressed PE-induced hypertrophic growth in NRVM. After being cultured for 6 h in serum-free DMEM, myocytes were incubated with 2-APB for 10 min, or chelerythrine (Chelery, PKC inhibitor), or Chelery (3 μmol·$L^{-1}$)+2-APB (4 μmol·$L^{-1}$) for 20 min, or Xe C for 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 [$^3$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 $IP_3Rs$ 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 $IP_3R$ pathway is the exclusive mediator of the $\alpha_1$AR hypertrophic effects. Indeed, combined $IP_3R$ 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 $IP_3R$ and PKC pathways contribute to $\alpha_1$AR-mediated hypertrophic effects[6,9].

To obtain more direct evidence for the role of $IP_3R$ in initiating cardiac hypertrophic growth, we also tried $IP_3BM$ (10 $\mu mol \cdot L^{-1}$) 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 $IP_3R$ activation as a cellular mechanism underlying $\alpha_1$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 $IP_3$, $IP_3BM$ can enter into cells and release free $IP_3$ inside, enabling us to observe $Ca^{2+}$ signaling response to $IP_3R$ activation in intact cells. At the cellular level, $IP_3BM$ stimulation increases the frequency of spontaneous $Ca^{2+}$ transients to 3-fold (Figure 1). At the elementary release level, the action of $IP_3BM$ 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 $IP_3Rs$ in NRVMs (data not shown here). These similar responses in local as well as global $Ca^{2+}$ signaling to $IP_3BM$ and $\alpha_1$AR stimulation are all sensitive to inhibition of $IP_3Rs$, further confirming activation of $IP_3Rs$ as the key mechanism in $\alpha_1$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, $IP_3Rs$ 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-$IP_3R$-$Ca^{2+}$-CaMK II may converge to a signaling pathway at the site of the nucleus.

The observation that PE (data not shown) and $IP_3BM$ 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 $IP_3Rs$ in $\alpha_1$AR-stimulated NRVM growth. While previous studies have linked the hypertrophic responses to the DAG-PKC branch of the $\alpha_1$AR signaling cascades[9], a major finding of the present work is that $IP_3R$ inhibitors, at concentrations that effectively block $\alpha_1$AR- and $IP_3BM$-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 $\alpha_1$AR stimulation depended on the concurrent $IP_3$-$IP_3R$ 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 $IP_3R$ activation plays an important role in triggering local as well as global $Ca^{2+}$ release in $\alpha_1$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 $G\alpha_q/G\alpha_{11}$ 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, 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 $Ca^{2+}$-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, Molkentin 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 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 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 Molkentin 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.