Immunolocalization of type 2 inositol 1,4,5-trisphosphate receptors in cardiac myocytes from newborn mice

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INOSITOL 1,4,5-TRISPHOSPHATE (InsP3) receptors (InsP3Rs) and ryanodine receptors (RyRs) are intracellular calcium release channels. In cardiac muscle it has been established that the role of RyRs is to release calcium during excitation-contraction coupling. In contrast, the role of InsP3Rs in cardiac myocytes is not clear. It has been proposed that InsP3Rs are involved in intracellular signaling because they have been found in Purkinje myocytes of the conduction system at high levels (17) and in intercalated disks (25). InsP3Rs are also involved in intracellular signaling because InsP3 has been shown to elicit slow calcium release and activate contractions in skinned ventricular muscle or permeabilized cells (21, 24, 53), and they have been isolated from the sarcoplasmic reticulum of interventricular septum tissue (5). Interestingly, other studies have also suggested that InsP3Rs may play a signaling role related to cell growth because these receptors are the first release channels expressed in embryos and both InsP3-dependent and -independent pathways persist during development (35, 44).

The idea of InsP3-R involvement in cell growth is supported by experiments performed by other investigators in which long-term activation of signaling pathways that generate InsP3 results in expression of genes involved in cellular hypertrophy (3, 10, 14, 55). However, how cardiac cells discriminate between the InsP3-induced changes in calcium and the large and cyclic fluctuations of calcium during excitation-contraction coupling is unknown. Furthermore, it is also unknown how InsP3Rs may alter expression of genes during cell growth. The phenomenon of slow waves of calcium or calcium oscillations promoting expression of hypertrophic genes in other systems (8, 23) does not fit with the normal functioning of cardiac cells because they experience rhythmic elevations of calcium at a much faster rate. Thus cardiac myocytes must tightly regulate the factors that alter gene expression. A plausible explanation is that calcium release by InsP3Rs in cardiac myocytes occurs at discrete sites where it would have a direct effect on gene expression. To test this hypothesis, we investigated the localization of InsP3Rs, their ability to release calcium, and a possible regulatory function of these receptors on the expression of some of the hypertrophic marker genes.

The results reveal the previously unidentified presence of type 2 InsP3Rs associated with the nuclear region and in striations in cardiac myocytes. In addition, the data provide strong evidence in favor of type 2 InsP3R involvement in intracellular signaling and promotion of cardiac cell growth.

MATERIALS AND METHODS

Cell isolation. Experiments were approved by the Animal Care and Use Committee of the University of Illinois at Chicago and followed the guidelines of the National Institutes of Health. Primary cultures were prepared from cardiac muscle of postnatal day 0 mice (stock CACNA1SxNIHS-BCfBR) as described previously (1, 2). Mice were anesthetized by methoxyflurane inhalation and decapitated. Hearts were removed, and ventricles were isolated and finely minced. The pieces of muscle were incubated at 37°C for 30 min in calcium- and magnesium-free rodent Ringer solution [in mM: 155 NaCl, 5 KCl, 11 glucose, and 10 HEPES, pH 7.4, containing collagenase type IA (1 mg/ml; Sigma)]. Dissociated muscle was triturated with a Pasteur pipette in culture medium [90% (vol/vol) Dulbecco’s modified Eagle’s medium with 4.5 g/l glucose (DMEM), and 2.5% horse serum]. This suspension was filtered and centrifuged, and a suspension of single myocytes was obtained. Cells were either cultured or used immediately for immunoblotting. For tissue culture, cells were plated

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on collagen-coated glass coverslips or 35-mm culture dishes at a density of 1 × 10⁶ cells per dish. Cultures were maintained in a 37°C incubator in DMEM with 2% horse serum, 20 μM arabinosylcytosine, 10 U/ml penicillin, and 10 μg/ml streptomycin. After 24 h the medium was replaced with fresh medium without arabinosylcytosine. Endothelin-1 (Calbiochem) or phenylephrine (Sigma) was added and left for 48–72 h.

Confocal microscopy. Single cells were examined for InsP₃R localization and changes in calcium concentration with a laser scanning confocal system (Bio-Rad Radiance 2100) attached to an inverted microscope (Nikon Eclipse TE300) with a ×60, 1.2 numerical aperture water-immersion objective.

To detect InsP₃Rs, cells were fixed with 4% paraformaldehyde in phosphate-buffered saline (PBS) and permeabilized with 0.1% Triton X-100. Cells were then incubated in 10% goat serum in PBS for 2–3 h to block nonspecific labeling. Primary antibodies were diluted in PBS plus 0.1% Triton X-100. The anti-type 1 InsP₃R antibody (Affinity BioReagents) was used at a 1:250 dilution. For detection of type 2 InsP₃R we used an antibody against the amino terminal (Affinity BioReagents) or the carboxy terminal (Santa Cruz Biotechnology), both at a 1:200 dilution. The anti-type 3 InsP₃R antibody (Sigma-Aldrich) was also used at a 1:200 dilution. The secondary antibody carried an FITC derivative (Alexa Fluor 488; Molecular Technology), both at a 1:200 dilution. The anti-type 1 InsP₃R antibody (Affinity BioReagents) or the carboxy terminal (Santa Cruz Biotechnology) was used at a 1:750 dilution. After 2 h of incubation, the coverslips were washed with PBS and mounted under a coverslip containing confocal system (Bio-Rad Radiance 2100) attached to an inverted microscope (Nikon Eclipse TE300) with a ×60, 1.2 numerical aperture water-immersion objective.

Western blotting. Cellular fractions were separated from isolated neonatal cells with a subcellular proteome extraction kit (Calbiochem) following the manufacturer’s instructions for cells in suspension. The four fractions obtained (cytosol, membrane/organelle, nucleus, and cytoskeleton) were used immediately or stored at −80°C until use. Proteins were separated by SDS-polyacrylamide gel electrophoresis using 7.5% gels and transferred to polyvinylidene difluoride (PVDF) membranes. The membranes were incubated with the amino-terminal anti-type 2 InsP₃R primary antibody used for immunolocalization of InsP₃Rs with confocal microscopy at a 1:10,000 dilution. After washing and blocking, membranes were incubated with a secondary antibody conjugated to horseradish peroxidase at 1:20,000 dilution. Antibody binding was detected with chemiluminescence by using the SuperSignal West Femto Detection Kit (Pierce) according to the manufacturer’s instructions. To determine the purity of these fractions, blots were also examined with antibodies against specific proteins in each subcellular fraction: anti-calpain-1 (Affinity Bioreagents) for the cytosolic fraction, anti-sarc(endo)plasmic reticulum Ca²⁺/ATPase (SERCA)2a (Sigma) for the membrane/organelle fraction, anti-histone deacetylase 2 (Zymed Laboratories) for the nuclear fraction, and anti-desmin (Sigma) for the cytoskeleton fraction. These antibodies were also detected with chemiluminescence. The distributions of the signals in the cytosol, membrane/organelle, nucleus, and cytoskeleton fractions, respectively, for three determinations were as follows (means ± SE): calpain-1: 94 ± 2%, 4 ± 0.3%, 2 ± 0.6%, 0%; SERCA2a: 2 ± 0.6%, 95 ± 0.4%, 3 ± 0.9%, 0%; histone deacetylase 2: 0%, 1.7 ± 0.3, 98 ± 0.9, 0.7 ± 0.7%; and desmin: 3 ± 1.2%, 37 ± 0.3%, 3.3 ± 0.3%, 90 ± 0.9%.

RESULTS

Immunolocalization of InsP₃Rs. Localization of InsP₃Rs was studied in primary cultures of murine neonatal cardiac myocytes with confocal microscopy. InsP₃R types 1–3 have been found in mouse, rat, and ferret hearts (12, 29, 40, 51, 54). Although the proportions of each isoform vary from report to report, the consensus is that type 2 is the most prominent isoform in preparations of ventricular tissue and the only isoform detected in isolated neonatal cardiocytes (40). Thus the pattern of InsP₃R expression in our cardiac cell culture was investigated with antibodies against InsP₃R types 1–3. Sarcomeric actin and the nucleus were labeled with rhodamine phalloidin and TO-PRO-3, respectively. In agreement with a previous report using adult mouse hearts (12), the predominant species of InsP₃Rs found in our preparations were types 1 and 2. However, the distributions of these two isoforms were quite distinct. Type 1 InsP₃Rs were not detected in myocytes (Fig. 1, a–d), whereas they were abundant throughout the cytosol in nonmyocyte cells (Fig. 1, e–h). In contrast, type 2 InsP₃Rs appeared only in myocytes (Fig. 2). Type 3 InsP₃Rs were not detected in either myocytes (Fig. 1, i–l) or nonmyocytes (not shown). The unique presence of type 2 InsP₃Rs in mouse myocytes agrees with studies by Perez et al. (40) using isolated neonatal myocytes from rat and ferret, in which they also found only type 2 InsP₃Rs.

The intracellular localization of type 2 InsP₃Rs in cardiac myocytes was further explored and is shown in more detail in
Figs. 2 and 3. Examination of 354 cells from 8 different cultures using the antibody against the amino terminal revealed a striking disposition of type 2 InsP₃ Rs (Fig. 2, a–d). Type 2 InsP₃ Rs were preferentially found associated with nuclear staining. Type 2 InsP₃ Rs also colocalized with rhodamine phalloidin staining, creating a striated pattern characteristic of cardiac myocytes. However, as seen in the two adjacent cells in Fig. 2, a and b, type 2 InsP₃ Rs were not found in all areas of rhodamine phalloidin staining. Nevertheless, the striated pattern was also found in cells labeled only with the anti-type 2 InsP₃ Rs antibody (Fig. 2, e–h). Inspection of 205 cells labeled with the anti-type 2 InsP₃ R antibody alone demonstrated that 100% of these cells had type 2 InsP₃ Rs associated with the nuclear domain, whereas only ~45% of them (93 of 205 cells) also had colocalization of type 2 InsP₃ Rs labeling with rhodamine phalloidin. By comparison, ~80–90% of cells labeled with rhodamine phalloidin alone showed striations. This indicates that type 2 InsP₃ Rs are always associated with the nucleus but not with rhodamine phalloidin staining.

Localization of type 2 InsP₃ Rs in cardiac myocytes was also examined with an antibody against the carboxy-terminal portion of the protein. The distribution of type 2 InsP₃ Rs detected with the carboxy-terminal antibody was the same as that found with the amino-terminal antibody. Type 2 InsP₃ R was localized in association with nuclear staining in all cells labeled with the carboxy-terminal antibody [120 cells (100%) from 2 different cultures] and in some cases in association with rhodamine phalloidin staining [47 of 120 cells (39%)] (Fig. 2, k–n).

The images in Fig. 3 correspond to a representative cell labeled with the amino-terminal (first 4 rows) or the carboxy-terminal (last row) anti-type 2 InsP₃ R antibody that had only type 2 InsP₃ R staining associated with nuclear staining. The first four rows in Fig. 3 are a series of successive images taken from different planes of the cell at 0.5-µm increments, and they show a clearer relationship of the type 2 InsP₃ Rs with the nuclear staining. The last row in Fig. 3 was obtained with the carboxy-terminal antibody, and it demonstrates the same association of type 2 InsP₃ Rs with nuclear staining as that shown with the amino-terminal antibody. Thus the results obtained with antibodies to either the amino or the carboxy terminal of the type 2 InsP₃ R protein are indistinguishable. In addition, detection of type 2 InsP₃ R with the carboxy-terminal antibody suggests that the full-length protein is being expressed.

A low level of type 2 InsP₃ R staining was also detected throughout the cells in the form of discrete clusters.
inize the possibility of bleed-through of TO-PRO-3 staining or nonspecific background staining, control experiments were performed in which the primary antibody was omitted or preadsorbed with the peptide used to generate the antibody. Both controls gave identical results. Figure 2, i and j, shows the nuclei in the cells and the absence of type 2 InsP₃ R staining when the primary antibody was omitted. The consistent pattern of colocalization of type 2 InsP₃ Rs in the nuclear region in neonatal cardiac myocytes provides support for the putative involvement of these receptors in modulation of nuclear calcium and changes in gene expression.

**Detection of type 2 InsP₃ Rs in cellular fractions by Western blot.** Isolated cardiocytes were suspended, fractionated, and used to determine the subcellular location of type 2 InsP₃ Rs. Proteins were electrophoresed on 7.5% SDS-polyacrylamide gels and transferred to PVDF membranes. Incubation of the membranes with the antibody directed against the amino terminal of type 2 InsP₃ Rs showed a single signal with a molecular mass of ~260 kDa (Fig. 4), which corresponds to the expected molecular weight of the type 2 InsP₃ R (36). The immunoreactive signal was elicited in both the membrane/organelle and nuclear fractions. Interestingly, the signal intensity was stronger in the nuclear fraction than in the membrane/organelle fraction. There was no apparent signal in either the cytosolic or the cytoskeletal fraction. Detection of signals in the nuclear and membrane/organelle fractions agrees with the colocalization of type 2 InsP₃ Rs with the nuclear staining and rhodamine phalloidin staining, respectively, found by confocal microscopy. Furthermore, the strength of the signals seen in Western blots correlates with the fact that type 2 InsP₃ Rs are
Fig. 3. Serial sectioning of cardiac myocytes reveals the relationship of type 2 InsP$_3$R with the nuclear region. Cells were labeled as in Fig. 2. Images correspond to myocytes that had only type 2 InsP$_3$R staining in the nuclear region. The first 4 rows correspond to successive images collected every 0.5 μm in the z plane in a cell labeled with the antibody against the amino-terminal portion of the type 2 InsP$_3$R. The last row shows an example of the images collected with the antibody against the carboxy terminal of the protein. The scale bar represents 5 μm.
always found in association with nuclear staining but not with rhodamine phalloidin in neonatal cardiac cells.

Activation of type 2 InsP₃R-mediated calcium release. The functional significance of type 2 InsP₃R localization in the nuclear region and in the striations was investigated. Calcium release was measured with fluo 4 in cardiac myocytes permeabilized with saponin (32), labeled with TO-PRO-3 and rhodamine phalloidin, and in the presence of 100 μM tetracaine to block ryanodine receptors. The frequency of calcium release events was very low (~1–2 per min; n = 63 cells) in unstimulated myocytes at a free calcium concentration of 100 nM. Stimulation of type 2 InsP₃Rs with the InsP₃ analog adenophostin A (100 nM; Ref. 50) significantly increased the frequency of the events to 8.3 ± 2.4 events/min (mean ± SE; n = 38 cells). Interestingly, calcium release was detected in close association with the nucleus and in other areas of the cells, as demonstrated in the merged images shown in Fig. 5, a and b, respectively. The frequency of events was further increased when the level of free calcium was raised to 150 nM. The average frequency in 150 nM calcium was 12.2 ± 1.8 events/min (n = 24 cells). Additionally, the higher calcium concentration also induced the generation of calcium waves. These experiments demonstrate that, as suspected, calcium release occurred at sites coincident with the immunolocalization of type 2 InsP₃Rs, underscoring an emerging key role of these receptors in cell signaling.

To further demonstrate that type 2 InsP₃Rs mediated the release events, calcium release was recorded from saponin-permeabilized myocytes without actin staining and before and after the addition of InsP₃R antagonists. Recordings were initially performed in the presence of adenophostin A, 100 nM calcium, and 100 μM tetracaine. Figure 5c shows two calcium events that occurred simultaneously in the same cell. One of the events localized toward the center of the nuclear region and the other close to the periphery of the nucleus. The events recorded by line scan under these conditions had an average amplitude of 0.9 ± 0.08 F/F₀, a duration of 112.4 ± 7.4 ms, and an average width of 3.2 ± 1.1 μm measured at 50% of the maximum amplitude (n = 152 events). These values fall within the range of those measured in a wide variety of cells (4, 38, 49), including cardiac myocytes (56). As shown in Fig. 5d, the calcium release events completely disappeared immediately after the cells were exposed to the InsP₃R blockers 2-APB (40 μM) or XeC (100 μM), clearly confirming mediation by InsP₃Rs.

Potential modulation of gene expression by type 2 InsP₃Rs. To determine the role of calcium release in the nuclear domains, we measured gene expression of some of the molecules that are affected when signaling cascades involving InsP₃Rs are activated, such as atrial natriuretic factor (ANF) and skeletal α-actin (3, 13, 14, 48). Gene expression was measured with RT-PCR. The RNA message was normalized to expression of GAPDH measured concurrently with each gene examined, and results from test conditions were normalized to the paired controls. Equal amounts of RNA were used in each RT-PCR assay. Cardiac cells were exposed to the agonists endothelin-1 (1 μM) or phenylephrine (10 μM) for 48–72 h to stimulate the hypertrophic response. Endothelin-1 and phenylephrine significantly increased the expression of both ANF and skeletal α-actin genes (Fig. 6). The average increase of ANF and skeletal α-actin message with endothelin-1 was 2.05 ± 0.3 (n = 9 cultures) and 1.87 ± 0.32 (n = 6 cultures) times over control conditions, respectively (P < 0.05). The corresponding average increase with phenylephrine was 1.90 ± 0.35 (n = 7 cultures) and 1.65 ± 0.28 (n = 6 cultures) times over control, respectively (P < 0.05). Although the expression of ANF and skeletal α-actin was significantly higher in the presence of the hypertrophic agonists than under control conditions, the increase is smaller than that found in previous studies using rat cardiac myocytes (3, 13, 14, 26), which most likely is a reflection of the difference in species (11, 45). Furthermore, it

Fig. 4. Analysis of InsP₃R protein in different subcellular fractions. The presence of InsP₃Rs in subcellular fractions was investigated by SDS-PAGE and immunoblotting. Isolated cardiocytes from neonatal mice were fractionated into the four cellular fractions indicated at top. A clear signal was detected in the membrane/organellar and nuclear fractions. The intensity of the signal was stronger in the nuclear fraction. No signal was evident in either the cytosolic or the cytoskeleton fraction. The immunoreactive signals in the membrane/organellar and nuclear fractions were eliminated when the primary antibody was preadsorbed with the peptide used to generate the antibody (not shown). The markers correspond to the Precision Plus Protein Standard (Bio-Rad).

Fig. 5. Detection of calcium release. Calcium release events were recorded in association with the nucleus (a) or toward the periphery (b) in saponin-permeabilized myocytes, as indicated by the arrows. Cells were labeled with rhodamine phalloidin and TO-PRO-3. Calcium was detected with fluo 4. c and d: Permeabilized myocyte in the presence of adenophostin A and 100 nM calcium and fluo 4. Calcium release events occurred associated with the nuclear region (c, arrows) and were blocked by 2-aminoethoxydiphenyl borate (2-APB; d), indicating that the events were mediated by InsP₃Rs. F, fluorescence intensity of event; F₀, baseline fluorescence.
should be noted that hypertrophy of mouse myocytes in response to receptor activation in vitro has been seen by some investigators (27, 34, 37, 42, 52) but not by others (11, 45), probably reflecting differences in culture conditions. In our experiments, exposure of the myocytes to 2-APB (40 μM) or XeC (20 μM) resulted in a marked inhibition of expression of these two genes. It has been reported that 2-APB and XeC can affect the calcium transporters SERCA2 and RyR in addition to InsP3R and result in depletion of the endoplasmic reticulum in other cell types (6, 18, 22, 39, 41, 47). To determine whether the InsP3R antagonists had deleterious secondary effects in neonatal myocytes at the concentrations used here, we treated myocytes with thapsigargin (1–2 μM) to block SERCA2 and deplete the sarcoplasmic reticulum of calcium. However, these conditions were detrimental for the cells because they detached from the culture dishes and did not last for the duration of the experiment. We also exposed myocytes to tetracaine and found that it did not prevent the change in gene expression promoted by endothelin-1 or phenylephrine. Thus our results suggest that InsP3Rs mediate the increase in mRNA levels.

Unexpectedly, the reduction in mRNA levels by the InsP3R blockers was of a magnitude sufficient to suggest that InsP3R activation is critical to the basal expression of ANF and skeletal α-actin. To test this hypothesis, myocytes were treated with the blockers in the absence of InsP3R stimulation by endothelin-1 or phenylephrine. A dose-dependent decrease in the level of ANF and skeletal α-actin was observed in those cells exposed to 2-APB or XeC (Fig. 7). Concentrations of 40 and 100 μM 2-APB reduced the levels of ANF mRNA to 0.76 ± 0.07 and 0.2 ± 0.08 of control values (n = 8 cultures), respectively. Levels of ANF were 0.39 ± 0.10 and 0.44 ± 0.09 of control (n = 5 cultures) in the presence of 20 and 40 μM XeC, respectively. Similar values were obtained for skeletal α-actin with both blockers. The reduction in mRNA levels was specific for the two hypertrophic markers examined here, because GAPDH levels were not affected by the two hypertrophic agents (Fig. 6). As an additional control, we verified that L-type calcium channel subunit α2δ1-C expression was not modified by treatment with either endothelin-1 or the InsP3R blockers (Fig. 7d). The average levels of α2δ1-C subunit were 0.95 ± 0.11 and 1.07 ± 0.12 of control in the presence of endothelin-1 and endothelin-1 plus 2-APB, respectively. Thus these experiments corroborate the specificity of InsP3Rs in modulating expression of some hypertrophic genes.

FIG. 6. InsP3 Rs mediate increase in gene expression promoted by endothelin-1 and phenylephrine. a: Detection of atrial natriuretic factor (ANF; top band) and GAPDH in cells treated for 72 h with endothelin-1. Exposure to endothelin-1 significantly increased the relative expression of ANF RNA message, which was blocked by 2-APB. Lane 1, DNA marker; lane 2, untreated cells; lane 3, endothelin-1-treated cells; lane 4, cells treated with endothelin-1 and 2-APB. b and c: fold increase in ANF (b) and skeletal (Sk) α-actin (c) RNA message in cells treated with endothelin-1 or phenylephrine in the absence or presence of 2-APB. C, control; E, endothelin-1; PE, phenylephrine; A, hypertrophic agonist + 2-APB.

FIG. 7. InsP3R antagonists decrease levels of hypertrophic marker gene mRNA. a: ANF expression was decreased in myocytes treated with 2-APB alone. Lane 1, DNA marker; lane 2, untreated cells; lane 3, 40 μM 2-APB; lane 4, 100 μM 2-APB. b and c: Reduction of ANF expression with different concentrations (in μM) of the InsP3R blockers 2-APB (b) and xestospongin C (XeC; c) as indicated below bars. d: Levels of α2δ1-C calcium channel subunit were not modified by endothelin-1 or endothelin-1 + 2-APB, indicating the preferential influence of InsP3Rs on expression of the hypertrophic genes ANF and skeletal α-actin. C, control; E, endothelin-1; A, endothelin-1 + 2-APB.
experiments differs from the previous reports by De Smedt et al. (12) and Tamura et al. (51) mentioned above. This apparent discrepancy may be due to possible developmental differences between neonate and adult ventricular myocytes.

The sole presence of type 2 InsP₃Rs in neonatal mouse myocytes found here agrees with data obtained with isolated neonate rat ventricular myocytes in which type 2 InsP₃R is the only isoform found with RT-PCR (40) and suggests that this isoform is a critical component of intracellular signaling and the expression of hypertrophic genes. It was suggested previously that type 1 InsP₃Rs are involved in intercellular signaling because they have been identified in cardiac cells at the intercalated disk by immunogold electron microscopy (25) and in the sarcoplasmic reticulum of cells from the conduction system (17). Here we determine the localization of InsP₃Rs and provide support for a regulatory role of type 2 InsP₃Rs in gene expression in neonatal cardiac cells. Using two antibodies that have been shown to be specific for the type 2 InsP₃R (7, 20, 28, 30, 43), we demonstrate with confocal microscopy that these receptors are preferentially localized in the region of the nucleus and less frequently in association with the sarcomeres. Analysis of type 2 InsP₃R protein in subcellular fractions with SDS-PAGE and immunoblotting also demonstrates that type 2 InsP₃Rs are found in the membrane/organelle and nuclear fractions, which supports the data obtained with confocal microscopy.

Although the goal of this study was to determine the localization and function of InsP₃Rs in cardiac myocytes, the results supply additional and important information on the cell biology of neonatal mouse cardiac myocytes. The vast majority of studies involving signaling cascades in cardiac myocytes in vitro have been delineated by work done mostly with primary culture of neonatal rat ventricular cells. Even though rat cells have provided a wealth of valuable information, differences in signaling cascades between neonatal rat and neonatal mouse cardiac myocytes have been reported, most notably by Steinberg’s (45) and Simpson’s (11) labs. However, it should be noted, parenthetically, that there are also conflicting results between these two reports. For example, Sabri et al. (45) found that norepinephrine is ineffective in promoting hypertrophy and inositol phosphate accumulation in mouse myocytes. On the contrary, results from Deng et al. (11) showed that norepinephrine induced hypertrophy and that exposure to endothelin and phenylephrine promoted accumulation of inositol phosphates in mouse cells, which led them to conclude that aspects of acute signaling were intact in mouse myocytes. Our own experiments also show that the increase in ANF and skeletal α-actin mRNA is considerably smaller than that observed in rat myocytes. Although there are differences between signaling in neonatal rat and mouse myocytes, mice have become the preferred animal model to study cardiac hypertrophy because of transgenic technology. Results obtained from adult mice are compared with those from the historically preferred model of neonatal rat cells in culture. Thus it is not surprising to find that a correlation does not always exist between the data obtained from in vivo and in vitro experiments or from adult and neonatal cells. This discrepancy underscores the importance of examining the signaling cascades in neonatal mouse myocytes. The results obtained here show that neonatal mouse myocytes do experience changes in expression of at least some of the genes involved in hypertrophy on exposure of the cells to endothelin-1 and phenylephrine. More work is needed to determine whether type 2 InsP₃Rs modulate expression of other hypertrophic genes and to determine the distribution of InsP₃Rs in adult ventricular myocytes.

Despite the additional effects that 2-APB and XeC can have on various calcium transporters, our results suggest that the action of these antagonists on InsP₃R was mainly responsible for the blockade of the endothelin-1 and phenylephrine responses. In addition to preventing the effect of endothelin-1 and phenylephrine, the InsP₃Rs blockers also decreased the levels of ANF and skeletal α-actin in the absence of the agonists. These novel and exciting results suggest that InsP₃Rs are important for maintaining basal expression of these two genes, which are involved in growth and development in cardiac myocytes, and support the idea that type 2 InsP₃Rs are involved in the regulation of cellular responses such as growth and programmed cell death (19, 35, 44).

Although type 2 InsP₃Rs have also been found associated with the nucleus of other cell types (28), our work is the first to show that this calcium release channel is localized in association with the nucleus and the sarcomeres of cardiac myocytes. Quite possibly, these receptors are embedded in the tubular membrane-bound invaginations of the nuclear envelope proposed to exist initially by Lui et al. (31) and later described by Fricker et al. (15). Because the nuclear envelope and the sarcoplasmic reticulum are continuous, InsP₃R activation was expected to lead to calcium release in association with the nuclear region. Our work delineates a role for type 2 InsP₃Rs through localization, activation, and gene expression distinct from the role type 2 InsP₃Rs may have at the level of the sarcomeres. The separate functions provide a mechanism and support for the hypothesis that calcium concentration fluctuations occur within microdomains in single cells. These regional microdomains explain how an entity, e.g., calcium, can independently control numerous events in a single cell such as growth, development, and contraction.

GRANTS

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