Abnormal calcium and protein kinase C-ε signaling in hypertrophied atrial tumor myocytes (AT-1 cells)

RICHARD KLINE,1,2 TIANRONG JIANG,1 XIAOHONG XU,1 VITALYI O. RYBIN,1 AND SUSAN F. STEINBERG1,2

Departments of Pharmacology1 and Medicine2, College of Physicians and Surgeons, Columbia University, New York, New York 10032

Received 31 October 2000; accepted in final form 29 January 2001

Kline, Richard, Tianrong Jiang, Xiaohong Xu, Vitalyi O. Rybin, and Susan F. Steinberg. Abnormal calcium and protein kinase C-ε signaling in hypertrophied atrial tumor myocytes (AT-1 cells). Am J Physiol Heart Circ Physiol 280: H2761–H2769, 2001.—Cardiac hypertrophy leads to contractile dysfunction and altered hormone responsiveness through incompletely understood mechanisms. Atrial tumor (AT-1) myocytes (AT-1 cells) are a cardiomyocyte lineage that proliferates but hypertrophies when proliferation is prevented with mitomycin C. Because both states maintain a highly differentiated phenotype, AT-1 cells were used to explore the signaling pathways that accompany and/or contribute to hypertrophic cardiomyocyte growth. Mitomycin C-induced AT-1 cell enlargement is associated with a pronounced increase in the amplitude and the duration of both electrically stimulated calcium transients and endothelin receptor-dependent calcium responses. Studies with caffeine indicate that the intracellular pool of releasable calcium is similar in control and hypertrophied AT-1 cells. This agrees with the results of Northern analyses that show similar steady-state levels of transcripts encoding the sarcoplasmic reticulum Ca-ATPase (and higher levels of transcripts encoding the Na+/Ca2+ exchanger) in hypertrophied AT-1 cells, relative to proliferating control cultures. However, immunoblot analyses reveal a marked increase in the expression of protein kinase C (PKC)-ε (a critical intermediate in the signaling pathway for endothelin receptor-dependent modulation of intracellular calcium) during AT-1 cell hypertrophy; the abundance of other PKC isoforms is not changed. Collectively, these results identify reciprocal regulation between calcium/PKC signaling and hypertrophic growth. The evidence that AT-1 cell hypertrophy leads to abnormalities in calcium regulation and specific changes in PKC-ε signaling support the notion that pathophysiological changes in PKC-ε abundance lead to functionally important changes in hormonal modulation of cardiomyocyte function.

mitomycin C; sarco(endo)plasmic reticulum calcium ATPase-2; endothelin

CARDIAC HYPERTROPHY BEGINS as an adaptive response to hormonal signals or increased mechanical load. With intense or prolonged insults, hypertrophy becomes maladaptive, culminating in cardiac decompensation with prolongation of the cardiac action potential and defects in calcium homeostasis. β-Adrenergic receptor responsiveness characteristically also is impaired in hypertrophied or failing hearts (regardless of etiology); defects in β-adrenergic receptor signaling have come to be viewed as an additional cardinal manifestation of the functionally compromised heart (6). Responses to other hormonal stimuli (endothelin, angiotensin II, etc.) also are likely to be disordered. The signaling determinants for endothelin receptor responsiveness and the effects of hypertrophy in modulating responses to endothelin have not been subjected to a similar level of scrutiny.

Current knowledge of the molecular alterations that contribute to hypertrophy-induced contractile dysfunction and altered hormone responsiveness derives largely from studies in intact animal models. Such models generally reproduce the pattern of changes identified in human tissues and, at least in theory, permit samplings to identify early and longitudinal changes of the disease process. However, intact animal models are not optimally suited for discriminating changes that accompany the disease process from those that are etiologic. This is due, at least, in part, to the toxicity and/or loss of functional specificity of many of the selective signaling molecule inhibitors and ion channel blockers when they are used in intact animals (rather than in isolated cell models). Moreover, heart failure represents the end result of the integrated actions of a myriad of distinct signals. Distinguishing the role of any individual mechanism in the acquisition of structural, electrical, and/or functional elements of this complex disorder in an intact animal model represents a formidable challenge.

AT-1 cell cultures constitute a potentially unique experimental model to delineate the molecular pathways that contribute to morphological and functional cardiomyocyte remodeling (4). AT-1 cells were derived from right atrial tumors that developed in transgenic mice expressing the simian virus 40 large T-antigen driven by the atrial natriuretic factor (ANF) promoter (19). AT-1 cells can be serially propagated in vivo (by

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
provide novel evidence that disease-associated changes in PKC-e enhance endothelin receptor responsiveness attribut-

able to an increase in PKC-e expression. These results indicate that mitomycin C-treated AT-1 cells might constitute a pathophysiologically pertinent model to explore the determinants of hypertrophic signaling as well as the altered biology of hypertrophied atrial myocytes. This study demonstrates that AT-1 cell hypertrophy leads to changes in calcium cycling function as well as enhanced endothelin receptor responsiveness attributable to an increase in PKC-e expression. These results provide novel evidence that disease-associated changes in PKC-e expression might represent a key and fundamentally important mechanism to calibrate hormonal responsiveness in the heart.

METHODS

The AT-1 cell transplantable tumor lineage was maintained by sequentially passaging the cells into syngeneic host mice (B6D2/F1, female, Jackson Labs) (12, 19). Briefly, cells were isolated from tumor-bearing mice by anesthetizing the animals with isoflurane and removing the tumors aseptically. Tumors were minced well and then further dissociated by gentle agitation for 90 min at 37°C in the presence of 150 U/ml of collagenase (Sigma, St. Louis, MO), 200 U/ml of DNase, and 5% fetal bovine serum (FBS) (Hyclone, Logan, UT). The cell suspension was mixed 1:1 with complete medium containing penicillin (100 U/ml), streptomycin (100 µg/ml), 5% fetal bovine serum (Sigma), dexamethasone (1 nM), and insulin (16 µg/ml, GIBCO) followed by gentle centrifugation. The cells were washed twice in complete medium, preplated for 30 min to decrease contamination by other cells with proliferative capability such as cardiac fibroblasts, and then plated at a density of 5 × 10^5 cells/ml in 100-mm culture dishes (Primaria surface coating, plus fibronectin, bovine plasma, Sigma) with media changes every 2–3 days. Cell cycle arrest was accomplished by exposure to 30 µg/ml of culture grade mitomycin C (Sigma) for 6 h on culture day 2 or 3. A previous study (4) demonstrated that this concentration of mitomycin C completely inhibits proliferation (as determined by bromodeoxyuridine uptake and incorporation into DNA). Molecular and functional characterizations of hypertrophied AT-1 cells were performed 6–8 days after mitomycin C treatment of primary AT-1 cell cultures. Comparisons were between control (proliferating) AT-1 cell cultures maintained in culture for the same interval. All experiments were performed on primary cultures from tumors.

Cell surface area was measured by digitized image analysis. Images were captured with an Olympus M-2 inverted microscope (×20, phase contrast objective), digitized with a Dage-MTI CCD 72 black and white camera, and stored with a Targa Plus frame grabber. Offline analysis of cell surface area by planimetry was performed with ImageJ Software (National Institutes of Health, Bethesda, MD).

Immunoblot analysis for the abundance and subcellular distribution of PKC isoforms was performed as described previously. The comparisons of PKC abundance were performed on quiescent proliferating and mitomycin C-treated AT-1 cells; PKC isoform localization to caveolae would not influence these measurements because localization to caveolae represents only a minor fraction of total cell enzyme and occurs only after stimulation with agonist (endothelin or phorbol 12-myristate 13-acetate). Methods for the photometric measurement of intracellular calcium in cells loaded with the calcium-sensitive indicator fura 2 were performed previously. The comparisons of PKC abundance were performed on control (proliferating) AT-1 cell cultures maintained in culture for the same interval. All experiments were performed to show that the growth stimulatory effects of mitomycin C are pronounced and sustained in the course of the analysis to show that the growth stimulatory effects of mitomycin C are pronounced and sustained.

RESULTS

Mitomycin C promotes cell enlargement. A previous study showed that proliferation arrest with mitomycin C leads to AT-1 cell hypertrophy over the subsequent 2–4 days in culture (4). Figure 1 extends the time course of the analysis to show that the growth stimulatory effects of mitomycin C are pronounced and sustained. A single 6-h treatment with 30 µg/ml mitomycin C on the second day of culture causes a time-
dependent increase in cell size. The area subtended by individual cells is modestly, but significantly, increased by mitomycin C at day 7; the difference increases progressively over the subsequent 1–2 wk. Mitomycin C is reported to arrest proliferation without inducing any gross toxicity (4). Indeed, mitomycin C-treated cultures form confluent monolayer cultures that maintain vigorous, regular spontaneous contractile activity for 2–3 wk in culture without any evidence that mitomycin C impairs cell viability.

Mitomycin C-treated AT-1 cells display dysregulated calcium signaling. To determine whether mitomycin C-dependent AT-1 cell enlargement is accompanied by alterations in calcium handling, calcium transients in vehicle and mitomycin C-treated AT-1 cells were compared during continuous electrical stimulation at 0.5 Hz. Diastolic calcium is not different in control and mitomycin C-treated AT-1 cells. However, Fig. 2 shows that mitomycin C-treated AT-1 cells display a significant increase in both the amplitude of electrically driven calcium transients and an increase in their duration.

Altered intracellular calcium handling is a prominent feature of human heart failure and animal models of cardiac hypertrophy. In each case, prolongation of the relaxation kinetics of the calcium transient and the twitch has been attributed to impaired SR calcium reuptake function due to changes in the expression levels of key SR calcium transport proteins. Several studies report a decrease in SERCA2 mRNA levels, protein, and/or SR $^{45}$Ca$^{2+}$ reuptake function in human

Fig. 1. Mitomycin C (MMC) increases cell size. Cell size was assessed by planimetry of individual cells in high power fields for up to 2 wk after plating. Measurements were performed on 250 control (open circles) and MMC-treated cells (solid circles) from three different preparations (results are means ± SE). Lines are quadratic fits of all measured points.

Fig. 2. Electrically driven calcium transients in control and MMC-treated AT-1 cells. Right, representative tracings of signal-averaged calcium transients during continuous electrical field stimulation at 0.5 Hz. Left, quantification of the data from 35 control and 17 MMCs. The duration of the calcium transient was measured at half-maximal amplitude. Both amplitude and duration significantly increased in MMC-treated cells. *$P < 0.05$
heart failure [although the results of more detailed studies in animal models of hypertrophy are more discordant (2)]. To determine whether mitomycin C-induced changes in relaxation kinetics could be due to changes in SERCA2 expression, the relative abundance of SERCA2 transcripts was compared in control and mitomycin C-treated cells. Because there were no significant differences between GAPDH levels in proliferating and hypertrophied AT-1 cells, GADPH and 28S staining served as internal controls for gel loading and transfer. Figure 3 shows that the SERCA2 probe specifically hybridizes to a single 5.5-kb transcript. SERCA2 transcripts are slightly more abundant in mitomycin C-treated than in control AT-1 cells, but this difference is not statistically significant (NS, n = 5). In contrast, a Na+/Ca2+ exchanger probe specifically hybridizes to a single 7-kb transcript, which is significantly more abundant in the mitomycin C-treated cells than in the corresponding controls (157 ± 31%, P < 0.05, n = 5). Assuming coordinate regulation of mRNA and functional protein abundance, these results suggest that mitomycin C-induced hypertrophy does not alter SERCA2, but leads to an increase in Na+/Ca2+ exchanger expression.

Mitomycin C-treated AT-1 cells display augmented calcium responses to endothelin in association with increased expression of PKC-ε. Studies of the functional consequences of altered calcium regulation during hypertrophy and heart failure generally have focused on excitation-contraction coupling mechanisms (2). Changes in calcium regulatory proteins also would be predicted to influence G protein-coupled receptor modulation of intracellular calcium. However, most studies to date have focused on β-adrenergic receptors, where changes in calcium regulation are more likely to be attributed to hypertrophy/failure-dependent changes in the expression of cell surface β-adrenergic receptors, G protein subunits, and adenylyl cyclase rather than target calcium regulatory proteins. Because proximal components of the endothelin receptor signaling pathway generally are reported to be grossly unaffected by processes that lead to cardiomyocyte hypertrophy/failure ([7, 9] and previous studies [10] identified an effect of endothelin, which is to elevate intracellular calcium in AT-1 cells), the next studies compared calcium responses to endothelin in control and mitomycin C-treated AT-1 cells. Figure 4 shows that endothelin elevates intracellular calcium in control AT-1 cells (0.25 ± 0.04 ratio units), and that this response is strikingly more pronounced in mitomycin C-treated AT-1 cells (3.4 ± 0.8 ratio units). The endothelin-dependent increase in calcium also is more sustained in the mitomycin C-treated AT-1 cells than in controls (duration at half-maximal amplitude: control, 15 ± 3 s; mitomycin C, 54 ± 7 s; P < 0.05, n = 6 for each).

There is relatively little information on the mechanism(s) for endothelin-dependent changes in intracellular calcium. To begin to distinguish alternative mechanisms that could account for a bigger and more sustained calcium response to endothelin in hypertrophied AT-1 cells (and distinguish the relative contribution of calcium mobilization from intracellular stores vs. calcium entry through the sarcolemma), control and mitomycin C-treated cells were exposed to caffeine and then to endothelin under conditions where the SR calcium stores were depleted by prior caffeine application (Fig. 5). These records illustrate two important findings. First, the amplitude of the caffeine-induced calcium transient is equivalent in control and mitomycin C-treated AT-1 cells. This suggests that hypertrophy does not grossly alter the size of the releasable intracellular SR calcium pool (and is consistent with the results of Northern analysis, which do not detect any changes in SERCA2 mRNA levels). Second, the

---

**Fig. 3.** MMC-induced hypertrophy leads to an increase in the abundance of Na+/Ca2+ exchanger without changes in sarcoplasmic reticular Ca2+-ATPase (SERCA2) mRNA levels. RNA from control and MMC cultures was size fractionated and sequentially hybridized with cDNA probes for the sarcoplasmic reticular (SR) Ca-ATPase, the sarcolemmal Na+/Ca2+ exchanger, and glyceraldehyde 3-phosphate dehydrogenase (GAPDH). Results are from two representative separate paired proliferating control (C) and MMC cultures.
effect of endothelin to elevate intracellular calcium is prevented by the prior application of caffeine. These results provide evidence that the effect of endothelin to elevate intracellular calcium involves release of calcium from intracellular stores.

Because mitomycin C-dependent changes in calcium responses to endothelin cannot readily be attributed to changes in the releasable intracellular calcium pool, studies then examined more upstream components in the endothelin receptor pathway. Changes at the level of the endothelin receptor and its activation of phospholipase C were excluded by biochemical studies that showed a similar stimulation of inositol phosphate accumulation in proliferating (3.2 ± 0.3-fold over basal) and hypertrophied (2.9 ± 0.4-fold over basal) AT-1 cells exposed to 100 nM endothelin for 30 min [according to the protocols published previously, n = 6, NS (10)]. Hence, the focus shifted to PKC, which was implicated in the endothelin-induced rise in intracellular calcium in proliferating AT-1 cells in previous

![Fig. 4. Endothelin-dependent calcium responses in control and MMC-treated AT-1 cells. Superfusion was with 100 nM endothelin. It should be noted that the difference in the y-axis between the two panels only serves to underestimate the difference in the magnitude between these responses.](image)

![Fig. 5. Caffeine-induced calcium transients are equivalent in control and MMC-treated AT-1 cells; endothelin-dependent calcium responses are blocked by caffeine in control and MMC-treated AT-1 cells. Representative tracings showing the effect of superfusion with 10 mM caffeine followed by endothelin in control and MMC-treated AT-1 cells. The characteristics of the caffeine-induced calcium transient were similar in control and MMC-treated AT-1 cells [amplitude: 1.4 ± 0.3 ratio units, control; 1.5 ± 0.3 ratio units, MMC; duration at half-maximal amplitude: 29 ± 3 s, control; 34 ± 6 s, MMC: n = 7 each, not significant (NS)].](image)
studies. Further experiments revealed a marked inhibition in the increase in intracellular calcium in response to endothelin in mitomycin C-treated AT-1 cells pretreated with chelerythrine (0.4 ± 0.3 ratio units) or GF1092003X (0.2 ± 0.3 ratio units), relative to control cultures without PKC inhibitor (3.4 ± 0.8 ratio units; incubation with PKC inhibitors was at 10 μM for 20 min at 37°C; n = 6 for each). The inhibitory effects of two structurally distinct PKC inhibitors identify a role for PKC in the pathway linking endothelin receptor activation to a rise in intracellular calcium in mitomycin C-treated AT-1 cells.

AT-1 cells express three PKC isoforms: PKC-α, PKC-ε, and PKC-ζ. Previous studies in proliferating AT-1 cells implicated PKC-ε in the endothelin receptor-dependent pathway leading to a rise in intracellular calcium in proliferating AT-1 cells (10). Immunoblot analysis was performed to identify the PKC isoform(s) activated by the endothelin receptor in the mitomycin C-treated AT-1 cell counterparts. Figure 6 shows that both proliferating and mitomycin C-treated AT-1 cells express PKC-α and PKC-ε. PKC-α immunoreactivity largely is soluble in the basal state, whereas PKC-ε immunoreactivity is detectable in both soluble and particulate fractions. PKC-ε partitioning to the particulate fraction is not significantly altered by mitomycin C-treatment (65 ± 9% soluble in proliferating AT-1 cells; 58 ± 8% soluble in mitomycin C-treated AT-1 cells; n = 5). Endothelin induces a similar rapid and sustained translocation of PKC-ε from the soluble to the particulate fraction of proliferating and mitomycin C-treated AT-1 cells (84 ± 9% and 78 ± 10% increases in PKC-ε immunoreactivity in the particulate fraction at 1 min after exposure to endothelin in proliferating and mitomycin C-treated AT-1 cells, respectively, Fig. 6A). There is no subcellular redistribution (activation) of the calcium-sensitive PKC-α in either preparation, despite robust elevations of intracellular calcium in both. Although PKC-α activation without translocation cannot be formally excluded by these experiments, these results strongly suggest that the effect of endothelin to activate PKC is confined to the ε isoform in both proliferating and mitomycin C-treated AT-1 cells. Immuno blot analysis also consistently revealed higher levels of PKC-ε immunoreactivity in mitomycin C-treated AT-1 cells relative to proliferating control AT-1 cells (Fig. 6B). The increase in PKC-ε without a concomitant change in the abundance of PKC-α and PKC-ζ argues strongly that

Fig. 6. Endothelin induces the selective translocation of protein kinase C (PKC)-ε from the soluble to the particulate fraction of proliferating and MMC-treated AT-1 cells, which express higher levels of PKC-ε. A: AT-1 cells were incubated without or with 100 nM of endothelin for the indicated interval and then partitioned into soluble and particulate fractions in the presence of EGTA. Soluble and particulate fractions (60 μg/lane) were resolved by SDS-PAGE, transferred to nitrocellulose, and probed with PKC-ε and PKC-α-specific antibodies. Immunoblots are representative of 3 separate experiments performed on separate culture preparations. B: extracts from proliferating and MMC-treated AT-1 cells were partitioned into soluble (S) and particulate (P) fractions (that contain all of the cellular PKC immunoreactivity). Fractions were probed with antibodies that discriminate between the proteins; the antiserum raised against PKC-ζ is known to also cross-react with PKC-α. Immunoreactivity in the soluble and particulate fractions was summed for comparisons of PKC in control and MMC cultures. Right: representative experiment. Left: averaged results from 5 separate culture preparations. The amount of total cell protein recovered in the particulate fractions was similar in control (35 ± 2%) and MMC (34 ± 3%) AT-1 cells. (*P < 0.05)
changes in PKC-ε levels cannot be attributed to a nonspecific variable, such as a general change in cell size or protein composition induced by hypertrophy (i.e., is not related to the denominator used to express the results). Although the data could be normalized to other denominators (including total cell protein and total particulate protein) that would influence the absolute values for PKC isoform abundance, currently there are no data to justify the biological relevance of one denominator over another. Importantly, the choice of denominator would not qualitatively alter the conclusion that PKC-ε levels become elevated relative to other PKC isoforms in hypertrophied cardiomyocytes. Collectively, these results indicate that mitomycin C-dependent hypertrophy leads to enhanced PKC-ε expression and that these changes in PKC-ε abundance are associated with a functionally important increase in endothelin-dependent modulation of intracellular calcium.

DISCUSSION

Many animal models have been used as surrogates to study the changes in calcium regulation and signaling proteins that develop during human cardiac hypertrophy and failure. Although these intact animal models circumvent many of the limitations inherent in studies of human tissues, none offers the obvious advantages of economy, flexibility, and control inherent in a cell culture model. The available intact animal and cell culture models also largely have been developed to investigate hypertrophy of the ventricle. Yet, the most common arrhythmia encountered in clinical practice is atrial fibrillation, an arrhythmia perpetuated by its propensity to structurally and electrically remodel the atrium. Because AT-1 cells hypertrophy while retaining features characteristic of differentiated atrial myocytes, they are intrinsically superior to other models as a surrogate to explore mechanisms underlying hypertrophic growth of atrial tissue. AT-1 cells are homogeneous, highly differentiated cardiomyocytes that can be propagated or induced to hypertrophy in culture, permitting detailed kinetic analyses of the intracellular signaling pathways that accompany and/or contribute to cardiomyocyte growth. This study provides new information on the mechanism for regulation of intracellular calcium by endothelin and demonstrates that AT-1 cell hypertrophy leads to functionally important changes in the regulation of intracellular calcium, PKC isoform signaling, and endothelin responsiveness.

A major finding of this study is that AT-1 cell enlargement is associated with an increase in the amplitude and prolongation of the duration of electrically stimulated calcium transients. Prolongation of calcium transient relaxation kinetics is prominent in various human heart failure syndromes and animal models of cardiac hypertrophy. Defective calcium removal generally has been attributed to reduced levels of SERCA2 and impaired SR calcium uptake function (2). However, the changes in calcium transient kinetics occur without evidence of a defect in SERCA2 expression in hypertrophied AT-1 cells. Although an increase in PKC-ε expression could in theory lead to changes in SERCA2 phosphorylation/activation in the basal state, other mechanisms also must be considered. Phospholamban (that in its dephosphorylated form lowers the apparent affinity of SERCA2 for calcium and regulates the rate and amount of calcium sequestered by the SR) is variably reported to be influenced by some forms of hypertrophy or heart failure syndromes (13, 16, 21). However, it is unlikely that phospholamban influences calcium cycling function in AT-1 cells, because phospholamban is not detected in atrial myocytes [including AT-1 cells (11)]. Another consistent feature of many hypertrophy models is prolongation of the action potential duration, which can delay the kinetics of the action potential-stimulated calcium transient even without any inherent abnormality in calcium transport mechanisms. The cellular basis for action potential duration prolongation can include an increase in a depolarizing current, a reduction in repolarizing $K^+$ currents [transient outward $K^+$ current ($I_{to}$) and inward rectifier $K^+$ current ($I_{kr}$) (3)] or other mechanisms (1). AT-1 cells represent a good model to investigate the effects of hypertrophy on action potential duration, because their repolarizing currents have been characterized in detail (23). Finally, although generally not considered, the altered topology of hypertrophied cardiomyocytes, with a decrease in surface-to-volume ratio, might in itself be sufficient to impair calcium relaxation kinetics (in the absence of any structural and/or functional defects in SR or sarclolemmal membrane calcium regulatory processes).

The Na$^+$/Ca$^{2+}$ exchanger characteristically is increased in failing human hearts and in several animal models of hypertrophy (20). Because upregulation of the Na$^+$/Ca$^{2+}$ exchanger generally accompanies a reduction in SERCA2 levels (and/or SR calcium reuptake function), changes in Na$^+$/Ca$^{2+}$ exchange have been considered compensatory as an adaptive mechanism to shift the burden of calcium removal from reuptake at the SR to extrusion at the plasma membrane. However, the possibility that both might occur independently, as part of a fundamental program of heart failure-induced changes in excitation-contraction coupling function, has never been excluded. In fact, these studies suggest that changes in SERCA2 levels need not precede changes in Na$^+$/Ca$^{2+}$ exchanger expression. Although changes in Na$^+$/Ca$^{2+}$ exchanger expression do not appear to be compensatory to a defect in SERCA2 expression, increased forward mode Na$^+$/Ca$^{2+}$ exchanger activity would be predicted to contribute to diastolic calcium removal from the cytosol; this would mitigate the lesion in calcium regulation acquired during hypertrophy of AT-1 myocytes. However, Na$^+$/Ca$^{2+}$ exchange can also operate in the reverse mode. Here, Na$^+$/Ca$^{2+}$ exchange could provide a source of activator calcium to trigger additional calcium release from the cardiac SR and supply inotropic support. In fact, the increased reverse mode Na$^+$/Ca$^{2+}$ exchange might at least, in part, explain the increased calcium
transient amplitude observed in mitomycin C-treated AT-1 cells.

Endothelin acts through a G protein-coupled receptor to elevate intracellular calcium, enhance contractile performance, and stimulate cardiomyocyte growth. There is only limited information on the mechanism(s) underlying the endothelin-dependent rise in intracellular calcium in cardiomyocytes. Endothelin variably is reported to enhance calcium entry through T-type calcium channels in cultured neonatal rat ventricular cardiomyocytes (8) or mobilizes calcium from a caffeine and ryanodine-insensitive intracellular pool in rat atrial cells (22). Whereas AT-1 cells have an unusually high relative density of T-type calcium channels (21), these changes are etiologic (rather than accompanying) the consequences of cell enlargement, activation of hypertrophic signaling pathways, and changes in hemodynamic function were not discriminated. In contrast, this study provides novel evidence that endothelin-dependent signaling to calcium is exaggerated in hypertrophied cardiomyocytes. Because the caffeine-sensitive releasable pool of intracellular calcium is equivalent in proliferating and hypertrophied cardiomyocytes, the calcium response must be calibrated by an upstream component of the signaling pathway. Although subtle changes in multiple elements in the signaling pathway could be contributory, this study identifies a major change in PKC-ε. PKC-ε is a necessary component of the pathway for endothelin receptor mobilization of intracellular calcium in both proliferating and hypertrophied AT-1 cells [Ref. 10 and results reported here]. The observation that PKC-ε abundance increases in hypertrophy in association with a more robust endothelin receptor-dependent calcium response suggest that PKC-ε calibrates the cellular response to endothelin.

The rate-limiting molecular switches that translate cell surface mechanical or hormonal stimuli into a hypertrophic phenotype have been the focus of intense investigation. Although the abundance of certain signaling molecules is reported to differ between normal and hypertrophied cardiomyocytes, the extent to which these changes are etiologic (rather than accompaniments of the cell growth response) is difficult to discriminate in intact animal models. In this context, this study identifies changes in two key hypertrophic signals in mitomycin C treated AT-1 cells. First, hypertrophied AT-1 cells have elevated integrated calcium levels; calcium is reported to mediate hypertrophic signaling at least, in part, through the activation of specific calcium-dependent molecular targets such as calmodulin kinase II and calcineurin (15, 17). Second, hypertrophied AT-1 cells are enriched in PKC-ε. Previous studies established that PKC-ε plays a specific role in signaling to the extracellularly regulated kinase cascade in AT-1 cells; recent studies suggest that PKC-ε has a function in the normal postnatal maturational growth of cardiomyocytes (10, 14). The changes in PKC-ε expression identified in this study are relatively modest in magnitude (compared with the marked changes in protein abundance typically achieved in overexpression studies). However, there is recent evidence that mere manipulation of PKC-ε targeting to its substrates (without changing its expression level) is sufficient to functionally impact on cardiomyocyte growth (14). The studies reported here suggest that subtle hypertrophy-induced changes in PKC-ε expression are sufficient to impact on the regulation of intracellular calcium by endothelin receptors and support the notion that relatively modest elevations in PKC-ε expression associated with disease are functionally important.

This work was supported by National Heart, Lung, and Blood Institute Grant HL-28958 (to S. F. Steinberg), a Grant-in-Aid from the American Heart Association (to S. F. Steinberg), and a National Institutes of Health postdoctoral training grant in pharmacological sciences (Grants 072717 to T. Jiang) and a training grant in Hormones: Biochemistry and Molecular Biology, 2T32 DK07328-18 (to X. Xu).

REFERENCES


