The low molecular weight GTPase Rho mediates a variety of cytoskeleton-dependent cell functions and stretch- and Gq-protein-induced hypertrophic responses in cardiac myocytes. Although ROCK, one of Rho’s effectors, has been suggested to mediate hypertrophic signals, the relationship of Rho/ROCK with downstream signals is unknown. A zinc finger transcription factor, GATA-4, is activated by extracellular signal-regulated kinase 1/2 and is required for the up-regulation of the endothelin-1 gene during myocardial cell hypertrophy. However, it is unknown whether Rho/ROCK signals are linked to downstream GATA-4. By transient transfection assays using a dominant-negative mutant and an activated derivative of ROCK-I, we showed that ROCK-I participates in GATA-4-dependent endothelin-1 transcription. Inhibition of the Rho/ROCK pathway by Y-27632, a selective inhibitor of ROCK, suppressed phenylephrine-stimulated phosphorylation of extracellular signal-regulated kinase 1/2 and increased the DNA binding activity of cardiac GATA-4. Interestingly, latrunculin B, which inhibits actin polymerization, also prevents phenylephrine-induced responses. These findings demonstrate that the Rho/ROCK pathway is linked to downstream GATA-4 via the activation of extracellular signal-regulated kinases during myocardial cell hypertrophy. The results also suggest that changes in actin dynamics provide a convergence point for Rho/ROCK to the downstream signals during this process.

Cardiac myocytes within the adult heart are terminally differentiated and do not undergo cell division. In response to stimuli that affect the mechanical load on the heart or in response to various neurohumoral factors, the heart adapts through the activation of a hypertrophic response in individual cardiac muscle cells. This response is characterized by an increase in myocyte size, accumulation of contractile proteins within individual cardiac cells, and activation of embryonic gene marker expression (for reviews, see Refs. 1–3). For example, genes such as β-myosin heavy chain (MHC) and atrial natriuretic factor become highly expressed within ventricular myocytes (4–6). Studies focused on elucidating the mechanisms of transcriptional regulation of these genes have identified a group of DNA-binding factors that might mediate the nuclear response to hypertrophic stimuli. These factors include the GATA family of zinc finger transcription factors, which mediate transcriptional activation of the genes for β-MHC and angiotensin II type 1a receptor during pressure overload-induced hypertrophy in vivo (7–9). GATA factors are also required for the transcriptional activation of the endothelin-1 (ET-1) gene during the transition from compensation to heart failure (10). During the hypertrophic process, cardiac GATA-4 is directly or indirectly phosphorylated by extracellular signal-regulated kinase (ERK)1/2, which increases the DNA binding ability of GATA-4 (10). However, the relationship between ERKs/GATA-4 and upstream signaling pathways is unknown.

One of the important upstream signaling molecules that mediate hypertrophic responses is Ras-like small GTPase Rho. Rho plays a critical role in a variety of cytoskeleton-dependent cell functions including actin polymerization, F-actin bundling, myosin-based contractility, focal adhesion formation, and cytokinesis in other cell types (11–15). In cardiac myocytes, activated Rho has profound effects on myofibrillar organization and stimulates c-fos and atrial natriuretic factor expression (16, 17). Treatment of neonatal rat cardiomyocytes with C3 exoenzyme, which abrogates Rho’s effects, suppresses the expression of natriuretic peptide genes induced by ET-1, angiotensin II, and phenylephrine (16, 18). Furthermore, dominant-negative Rho attenuates the Goq- and Ras-induced transcriptional activation of the atrial natriuretic factor gene (19–21). These results demonstrate that Rho plays an important role in the modulation of cardiac gene expression in hypertrophy that is induced by signaling through Goq.

Recently, several targets of Rho have been identified, including protein kinase N, citron kinase, ROCK-I (p160ROCK), and ROCK-II (Rho kinase) (15, 22–25). Although the Rho/ROCK pathway has been suggested to mediate hypertrophic signals in cardiac myocytes (16, 18), the relationship between the Rho/ROCK pathway and downstream ERKs/GATA-4 is unknown. In the present study, we investigated the linkage between Rho/ROCK and ERK/GATA-4 pathways during myocardial cell hypertrophy.

**EXPERIMENTAL PROCEDURES**

### Immunocytochemistry and Measurement of Cell Diameter—Primary neonatal rat ventricular cardiac myocytes were pre-

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**The low molecular weight GTPase Rho mediates a variety of cytoskeleton-dependent cell functions and stretch- and Gq-protein-induced hypertrophic responses in cardiac myocytes. Although ROCK, one of Rho’s effectors, has been suggested to mediate hypertrophic signals, the relationship of Rho/ROCK with downstream signals is unknown. A zinc finger transcription factor, GATA-4, is activated by extracellular signal-regulated kinase 1/2 and is required for the up-regulation of the endothelin-1 gene during myocardial cell hypertrophy. However, it is unknown whether Rho/ROCK signals are linked to downstream GATA-4. By transient transfection assays using a dominant-negative mutant and an activated derivative of ROCK-I, we showed that ROCK-I participates in GATA-4-dependent endothelin-1 transcription. Inhibition of the Rho/ROCK pathway by Y-27632, a selective inhibitor of ROCK, suppressed phenylephrine-stimulated phosphorylation of extracellular signal-regulated kinase 1/2 and increased the DNA binding activity of cardiac GATA-4. Interestingly, latrunculin B, which inhibits actin polymerization, also prevents phenylephrine-induced responses. These findings demonstrate that the Rho/ROCK pathway is linked to downstream GATA-4 via the activation of extracellular signal-regulated kinases during myocardial cell hypertrophy. The results also suggest that changes in actin dynamics provide a convergence point for Rho/ROCK to the downstream signals during this process.**

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**The abbreviations used are: MHC, myosin heavy chain; ET-1, endothelin-1; PE, phenylephrine; luc, luciferase; CAT, chloramphenicol.**

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pared as previously described (9, 10, 26–28). The cardiac myocytes were grown on flask-style chambers with glass slides (Nalgen Nunc, Naperville, IL) and then stimulated with saline or 1.0 × 10⁻⁵ M of PE in serum-free medium for 48 h. The cells were then fixed with 3% formaldehyde in phosphate-buffered saline for 15 min at room temperature. Immunocytochemical staining for β-MHC was performed using the indirect immunoperoxidase or immunofluorescence method as previously described (27). As the primary antibody we used anti-β-MHC polyclonal antibody (NovoCastra Laboratories Ltd., Newcastle, UK) at a dilution of 1:50.

A total of 50 myocardial fibers were selected randomly from cardiac myocytes stained with anti-β-MHC antibody, and the diameters of these cells were measured semiautomatically with the aid of an image analyzer (LUXEX 3U; Nikon, Tokyo, Japan) as previously described (29). The shortest diameters of β-MHC-stained myocytes were measured at the level of the nucleus.

RNA Analysis—Northern blot analysis of 10 μg of total RNA was performed as described previously (7, 26, 28). An isoform-specific antisense deoxyoligonucleotide complementary to nucleotides 5854-5869 of the rat 3'–untranslated region (30) was used to detect β-MHC mRNA as described previously (7, 26). To detect ANF mRNA, we used a 202-bp rat ANF cDNA probe obtained by the reverse transcriptase-PCR (31). As controls, blots were also hybridized with rat glyceraldehyde-3-phosphate dehydrogenase (GAPDH) cDNA (nucleotides 170–577) (32). Amounts of mRNAs were quantified using a bioimaging analyser (BAS 2000; FUJIX, Tokyo, Japan).

Plasmid Constructs—Expression vectors encoding a dominant-negative mutant of p160ROCK (KD-IA) and an activated ROCK derivative (ROCK-A), both of which were constructed in pCAG mammalian expression vectors, were generous gifts of ROCK Contributes to GATA-4 Activation

Effect of Y-27632 on PE-induced Hypertrophic Response—An α₁-adrenergic agonist, phenylephrine (PE), can activate several independent features of myocardial cell hypertrophy (1–3, 10). To examine the role of ROCK in the PE-induced hypertrophic response we utilized a specific inhibitor of ROCK, Y-27632. The affinity of this agent for ROCK kinases is at least 20 times higher than that for the other Rho effector kinases, citron kinase and protein kinase N (34). To evaluate the effects of this specific ROCK inhibitor on the PE-induced hypertrophic response cardiac myocytes were stimulated with saline or 10⁻⁵ M PE in the presence or absence of 3 μM Y-27632 for 48 h. These cells were then stained with an antibody against β-MHC. As shown in Fig. 1A, brown signals, which indicated the presence of β-MHC, were observed in both saline- and PE-stimulated cardiac myocytes. Cardiac myocytes stimulated with PE displayed increases in cell size and myofibrillar organization as compared with saline-stimulated cells. A therapeutic level of Y-27632 (3 μM) largely inhibited such changes. As shown in Fig. 1B, the myocardial cell diameter was significantly smaller in cells treated with PE plus Y-27632 (bar 6) than in cells treated with PE alone (bar 4). Y-27632 also dose-dependently inhibited the increase of the cell diameter induced by endothelin-1 (compare bars 7, 8, and 9), another representative hypertrophic stimulus. However, Y-27632 alone did not affect the myocardial cell diameter in the basal (i.e. in saline-stimulated
cardiac myocytes, bars 2 and 3), suggesting that the inhibitory effect of Y-27632 is not simply due to toxicity. We then examined the effects of Y-27632 on the PE-induced expression of cardiac genes encoding β-MHC and atrial natriuretic factor, whose up-regulation is a well established marker for myocardial cell hypertrophy. As shown in Fig. 2, Y-27632 (3 μM) significantly inhibited the induction of β-MHC and atrial natriuretic factor gene expression by PE. However, Y-27632 alone did not affect the basal expression of these genes. The expression of the ubiquitously and constitutively expressed GAPDH gene was not affected by PE or Y-27632. Taken together, these data demonstrate that a therapeutic concentration of Y-27632 can selectively suppress the PE-induced hypertrophic response.

Y-27632 Suppresses Transcriptional Activation of the ET-1 Promoter by PE—The up-regulation of the expression of the ET-1 gene during myocardial cell hypertrophy is mediated, at least in part, at the level of transcription, and that transcriptional activation of the ET-1 promoter during hypertrophy requires an intact GATA element within this promoter (10). We next examined the effect of Y-27632 on PE-induced activation of ET-1 promoter activities. We transfected 2.0 μg of CAT cDNA driven by the 204-bp rat ET-1 promoter (pETCAT) into cultured neonatal rat cardiac myocytes. A small quantity of pRSVlac (0.1 μg) was co-transfected to normalize for transfection efficiency. Stimulation of these cells with PE increased the relative CAT expression from pETCAT by 2.97 ± 0.21-fold (n = 3) compared with saline-stimulated cells, which is in agreement with our previous report (10). Y-27632 significantly inhibited the PE-stimulated increase in ET-1 promoter activity (p < 0.05, 78% decrease compared with PE stimulation, 1.44 ± 0.04-fold compared with saline stimulation, n = 3) whereas Y-27632 alone did not affect the activity in saline-stimulated cardiac myocytes (1.36 ± 0.11-fold compared with saline stimulation, n = 3). These findings suggest that this agent specifically blocks α1-adrenergic signaling in cardiac myocytes.

ROCK-I Is Involved in GATA-4/5-dependent ET-1 Transcription—Our previous studies demonstrated that expression of GATA-4 or -5 can activate the ET-1 promoter activity in a sequence-specific manner (10). To determine whether ROCK-I participates in GATA-4/5-stimulated ET-1 transcription, we performed co-transfection experiments in COS7 cells, which lack all GATA factors (10, 28, 35). An expression plasmid encoding a CAT reporter driven by the 204-bp ET-1 promoter was co-transfected with an expression plasmid encoding GATA-4 or -5. As shown in Fig. 3, the expression of GATA-4 and -5 resulted in marked activation of the 204-bp ET-1 promoter in a dose-dependent manner, compatible with our previous report (10). Expression of a dominant-negative form of ROCK-I by co-transfecting KD-IA significantly inhibited the GATA-4- and GATA-5-stimulated ET-1 promoter activity. In contrast, co-expression of a constitutively active ROCK-I mutant by co-transfecting ROCKΔ3 resulted in further activation of the GATA-4- or 5-dependent ET-1 promoter activity. These findings support the idea that ROCK-I participates in GATA-4/5-dependent ET-1 transcription.

Y-27632 Inhibits PE-induced ERK Activation in Cardiac Myocytes—Our recent report demonstrated that the MEK-1/ERK pathway plays a role upstream of GATA-4 and that ERK activation is required for the PE-induced phosphorylation of GATA-4 and increase in its DNA binding activity in cardiac myocytes (10). To investigate the linkage between Rho/ROCK and ERK/GATA-4 pathways, we examined whether Y-27632 affects PE-induced ERK activation. Neonatal rat ventricular myocytes or NIH3T3 cells were preincubated with or without 3 μM Y-27632 for 1 h. Then PE was added, and the myocytes were further incubated at 37 °C. Activation of ERK1/2 was evaluated by Western blot analysis using an antibody that specifically recognizes the phosphorylated, active form of these enzymes. ERK1/2 were markedly activated after 15 min of PE stimulation (Fig. 4A, lane 2) compared with saline stimulation (Fig. 4A, lane 1) in both cardiac myocytes and NIH3T3 cells. In cardiac myocytes, Y-27632 inhibited PE-stimulated ERK1/2 activation in a dose-dependent manner (Fig. 4A, lanes 3 and 4 in cardiac myocytes). In contrast, Y-27632 inhibited the phosphorylation of ERK1/2 only mildly in NIH3T3 cells (Fig. 4A, lanes 3 and 4 in NIH3T3 cells). Thus, inhibition of ERK activation by Y-27632 might involve cardiac-specific signal transduction pathways.

Y-27632 Suppresses the PE-induced Increase in the DNA Binding Activity of Cardiac GATA-4—To determine whether Y-27632 modulates the DNA binding activity of GATA-4 in cardiac myocytes, EMSAs were performed. Nuclear extracts were prepared from neonatal cardiac myocytes stimulated with
PE in the absence or the presence of Y-27632 (3 μM) or treated with saline as a control. These extracts were probed with a radiolabeled double-stranded oligonucleotide containing the ET-1 GATA site. As shown in Fig. 5A, competition EMSAs revealed that a retarded band represented specific binding, as evidenced by the fact that it was competed out by an excess of unlabeled ET-1 GATA oligonucleotide (lane 3), but not by the same amount of an oligonucleotide containing the ET-1 GATA site with a mutation (lane 2). To further confirm that the retarded band represents an interaction of the probe with GATA-4, we performed supershift experiments. The retarded band was supershifted by anti-GATA-4 antibody (lane 5), but not by control mouse IgG (lane 4). The amount of the specific complex containing GATA-4 markedly increased in nuclear

**FIG. 2.** Northern analysis of the expression of endogenous β-MHC, ANF, and GAPDH genes. Neonatal rat ventricular cardiac myocytes were incubated with saline or PE (1.0 × 10^{-5} M) in the presence or absence of Y-27632 (3 μM) for 48 h. Blots containing total RNA (10 μg) from these myocytes were sequentially hybridized with an isoform-specific antisense deoxyoligonucleotide complementary to rat β-MHC mRNA, with a rat ANF cDNA, and with a rat GAPDH cDNA. A, a representative photograph; B, quantitative analysis. The data presented in B are the means ± S.E. from four independent experiments.

**FIG. 3.** ROCK-I participates in GATA-4/5-dependent ET-1 transcription. COS cells were transfected with 2.0 μg of pETCAT and 0.1 μg of pRSVluc (internal control), in addition to 0.4 or 1.2 μg of pcDNAG4, pcDNAG5, or pCMVβ-gal; 2.0 μg of KD-IA or pCMVβ-gal; 0.4 μg of ROCKΔ3 or pCMVβ-gal. The total amount of DNA was kept constant at 5.3 μg. The results are expressed as fold activation of the normalized CAT activity (CAT/luc) relative to that resulting from transfection with 3.2 μg of pCMVβ-gal without pcDNAG4, pcDNAG5, KD-IA, or ROCKΔ3. The data shown are the means ± S.E. from three independent experiments.
ROCK Contributes to GATA-4 Activation

**FIG. 4.** ROCK inhibitor Y-27632 blocked PE-induced ERK1/2 activation in rat neonatal cardiac myocytes but not in NIH3T3 cells. Cardiac myocytes and NIH3T3 cells were preincubated with or without Y-27632 for 1 h and subsequently stimulated with or without PE for 15 min for ERK activation. Activation of ERK was evaluated by Western blotting, and the data were quantified as described under “Experimental Procedures.” A, a representative photograph; B, quantitative analysis. The data presented in B are the means ± S.E. from three independent experiments.

**FIG. 5.** Y-27632 inhibited PE-stimulated increase in the cardiac ET-1 GATA binding activity. Cardiac myocytes were preincubated with or without 3 μM Y-27632 for 1 h and subsequently stimulated with saline or PE for 3 h, as indicated. Nuclear extracts (10 μg of protein) from these cells were probed with a radiolabeled double-stranded oligonucleotide containing the ET-1 GATA site in A and B and with that containing the p53-binding site in C. The arrows in A and B indicate specific band of ET-1 GATA element with cardiac GATA-4.

extracts from PE-stimulated myocytes (Fig. 5B, lane 2) compared with those from saline-treated cells (lane 1). Notably, the PE-stimulated increase in the ET-1 GATA binding activity was almost completely blocked by even 3 μM Y-27632 (Fig. 5B, lane 3). In contrast, p53 binding activities were not altered by PE nor PE plus Y-27632 (Fig. 5C). These experiments were repeated three times using independent preparations of cells and were found to be reproducible.

*Latrunculin B Prevents ERK/GATA-4 Activation by PE*—Recent reports (36) suggest that LIM kinase is one of substrates of ROCK and that actin treadmilling provides a convergence point for LIM kinase-induced signaling to the nucleus. Therefore, we examined whether actin dynamics play a role in PE-induced ERK/GATA-4 activation, acting through Rho/ROCK. For this purpose, we tested the effect of latrunculin B, which sequesters G-actin monomers, on ERK/GATA-4 activation. Neonatal rat ventricular myocytes were preincubated with or without 100 or 1000 nM latrunculin B for 1 h. Then PE was added, and the myocytes were further incubated at 37 °C. Activation of ERK1/2 was evaluated by Western blot analysis as described above. As shown in Fig. 6, latrunculin B inhibited PE-stimulated ERK1/2 activation in a dose-dependent manner (compare lanes 4, 5, and 6) although it did not affect the phosphorylation status at the basal (compare lanes 1, 2, and 3). Then we examined whether latrunculin B modulates the DNA binding activity of cardiac GATA-4. Nuclear extracts were prepared from neonatal cardiac myocytes stimulated with PE in the absence or the presence of latrunculin B (100 nM) or treated with saline as a control. These extracts were subjected to EMSA using the ET-1 GATA site as a probe. As shown in Fig. 7A, the PE-stimulated increase in the ET-1 GATA binding activity was almost completely blocked by latrunculin B although p53 binding activities were altered by neither PE nor PE plus latrunculin B (Fig. 7B). Thus, latrunculin B prevents PE-induced activation of ERKs and increases the DNA binding activity of cardiac GATA-4.

*Latrunculin B Suppresses Hypertrophic Responses*—Finally, we investigated whether alterations in actin dynamics contribute to the development of myocardial cell hypertrophy. Neonatal rat cardiac myocytes were stimulated with saline, 10^{-5} M PE, or 10^{-7} M ET-1 in the presence or absence of 30 nM latrunculin B for 48 h. These cells were then subjected to immunofluorescence with an antibody against β-MHC. As shown in Fig. 8A, cardiac myocytes stimulated with PE displayed in-
**Fig. 6.** Latrunculin B, which inhibits actin polymerization, blocked PE-induced ERK1/2 activation. Cardiac myocytes were preincubated with or without latrunculin B for 1 h and subsequently stimulated with or without PE for 15 min for ERK activation. Activation of ERK was evaluated by Western blotting, and the data were quantified as described under “Experimental Procedures.” A, a representative photograph; B, quantitative analysis. The data presented in B are the means ± S.E. from three independent experiments.

**Discussion**

ROCK is a member of the Rho family of small G proteins (Rho, Rac, and Cdc42). The activation of Rho induces the formation of stress fibers and focal adhesions in various cell types including cardiac myocytes (11–15). Furthermore, several lines of evidence indicate that Rho mediates hypertrophic signals in cardiac myocytes (16–21, 37–39). C3 transferase, a specific inhibitor of Rho, or a dominant-negative form of Rho, inhibited sarcomere organization, and induction of atrial natriuretic factor expression during myocardial cell hypertrophy. In addition, overexpression of constitutively activated Rho stimulates c-fos and atrial natriuretic factor gene expression in these cells. Several targets of Rho have been identified, including Rho kinase (ROCK-II), p160 ROCK (ROCK-I), citron kinase, and protein kinase N (15, 22–25). Using Y-27632, a specific inhibitor of ROCK, and KD-IA, a dominant-negative mutant of ROCK-I, the present study demonstrated that activation of the Rho/ROCK pathway contributes to several independent features of myocardial cell hypertrophy, including increase in cell size, sarcomere organization and induction of atrial natriuretic factor and β-MHC expression. Aoki et al. (40) have demonstrated that myosin light chain kinase mediates sarcomere organization during myocardial cell hypertrophy (40). Phosphorylation of myosin light chain kinase is regulated by myosin light chain phosphatase, one of the substrates of ROCK. Our findings are compatible with those of previous studies and provide further evidence for the role of the Rho/ROCK pathway in myocardial cell hypertrophy. However, these data do not rule out a possible role for other targets of Rho in myocardial cell hypertrophy. In fact, Morissette et al. (41) have demonstrated that protein kinase N regulates atrial natriuretic factor gene transcription in cardiomyocytes through a serum response element. Interestingly, the stimulation of atrial natriuretic factor gene expression by ROCK occurs in a serum response element-independent manner (18, 42). Therefore, ROCK and protein kinase N appear to mediate hypertrophic responses through separate pathways. Subcellular signals activated by hypertrophic stimuli finally reach the nucleus and activate a subset of transcription factors. The zinc finger transcription factor GATA-4 is one of the targets of hypertrophic signals that mediate transcriptional activation of genes encoding β-MHC, angiotensin II type 1 receptor, B-type natriuretic factor, and endothelin-1 in cardiac myocytes (7–10). The DNA binding activity of cardiac GATA-4 increases during the hypertrophic process. The present study demonstrated that the activation of ROCK-I is involved in GATA-4-dependent ET-1 transcription. In addition, inhibition of the Rho/ROCK pathway by Y-27632 abrogated the increase in the DNA binding activity of cardiac GATA-4 during hypertrophy. These findings suggest that the Rho/ROCK pathway plays a role upstream of GATA-4 during myocardial cell hypertrophy. Because ERK1/2 activation is required for the serine phosphorylation of cardiac GATA-4, which increases its DNA binding ability (10), we investigated whether Y-27632, a specific inhibitor of ROCK, affects the activation of ERKs. Y-27632 dose-dependently suppressed PE-stimulated...
phosphorylation of ERK1/2 in cardiac myocytes as well as the increase in the DNA binding activity of cardiac GATA-4. These findings suggest that the Rho/ROCK pathway contributes to the activation of ERK/GATA-4 during hypertrophic signaling in cardiac myocytes.

The question arises as to how ROCK signals are transduced to ERKs. To date, several substrates of ROCK/Rho kinase have been identified. These include myosin light chain, the myosin binding subunit of myosin light chain phosphatase, and LIM kinase (43–47). A recent report suggests that actin treadmilling provides a convergence point for LIM kinase-induced signaling to the nucleus (36). Therefore, we investigated whether actin dynamics play a role in PE-induced ERK/GATA-4 activation, acting through Rho/ROCK. Our data show that latrunculin B, which inhibits actin polymerization, also prevents the PE-induced increase in cell size as well as activation of ERK/GATA-4. These data show that regulation of actin dynamics is required for α1-adrenergic-induced hypertrophic signals in cardiac myocytes and suggest that actin treadmilling provides a convergence point for Rho/ROCK signaling to ERK/GATA-4. However, further studies are needed to clarify how ROCK is connected with ERKs.

Our study has shown a novel linkage between Rho/ROCK and ERK/GATA-4 pathways in myocardial cell hypertrophy. Y-27632 has been shown to be potentially useful for decreasing blood pressure and preventing cancer metastasis in vivo. The results of the present study raise the question of whether this agent might be useful in experimental models of heart failure in vivo. However, several possibilities should be taken into account when the data of this study are applied to heart diseases in vivo. First, as myocardial development is not complete at birth, differences may exist between neonatal and adult cardiac myocytes. Second, dis-associated myocytes in culture may behave in a different manner from myocytes in the organized heart in vivo. Third, stimulation of normal myocytes with α1-adrenergic agonist may not always mimic chronic hemodynamic overload in the diseased state. Recently, Sah et al. (48) reported generation of transgenic mice overexpressing constitutively activated forms of RhoA in the heart. These mice manifested pronounced atrial enlargement and a lethal phenotype, often preceded by generalized edema. In addition, cardiac activation of Rho resulted in increased ventricular expression of ANF and β-MHC genes, followed by ventricular dilation and dysfunction. Given the blockage of independent features of the hypertrophic response by physiological concentrations of Y-27632, it would be of considerable interest to pursue the possibility of the therapeutic use of this agent in this animal model and in heart failure in humans.

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