A β₁-adrenergic receptor CaM kinase II-dependent pathway mediates cardiac myocyte fetal gene induction

Carmen C. Sucharov, Peter D. Mariner, Karin R. Nunley, Carlin Long, Leslie Leinwand, and Michael R. Bristow. A β₁-adrenergic receptor CaM kinase II-dependent pathway mediates cardiac myocyte fetal gene induction. Am J Physiol Heart Circ Physiol 291: H1299–H1308, 2006. First published February 24, 2006; doi:10.1152/ajpheart.00017.2006.—β-Adrenergic signaling plays an important role in the natural history of dilated cardiomyopathies. Chronic activation of β-adrenergic receptors (β₁-AR and β₂-AR) during periods of cardiac stress ultimately harms the failing heart by mechanisms that include alterations in gene expression. Here, we show that stimulation of β-ARs with isoproterenol in neonate rat ventricular myocytes causes a “fetal” response in the relative activities of the human cardiac fetal and/or adult gene promoters that includes repression of the human and rat α-myosin heavy chain (α-MyHC) promoters with simultaneous activation of the human atrial natriuretic peptide (ANP) and rat β-MyHC promoters. We also show that the promoter changes correlate with changes in endogenous gene expression as measured by mRNA expression. Furthermore, we show that these changes are specifically mediated by the β₁-AR, but not the β₂-AR, and are independent of α₁-AR stimulation. We also demonstrate that the fetal gene response is independent of cAMP and protein kinase A, whereas inhibition of Ca²⁺/calmodulin-dependent protein kinase (CaMK) pathway blocks isoproterenol-mediated fetal gene program induction. Finally, we show that induction of the fetal program is dependent on activation of the L-type Ca²⁺ channel. We conclude that in neonatal rat cardiac myocytes, agonist-occupied β₁-AR mobilizes Ca²⁺ stores to activate fetal gene induction through cAMP independent pathways that involve CaMK.

β-ADRENERGIC SIGNALING plays an important antithetical role in the natural history of dilated cardiomyopathies (DCMs) exerting favorable compensatory effects on cardiac function and promoting the development and progression of the DCM phenotype (4, 6, 11, 13). Activation of β-adrenergic receptors (β₁-AR and β₂-AR) during periods of cardiac stress initially results in increases in heart rate and contractility, effectively improving cardiac output, but then ultimately harms the failing heart (5, 8) by mechanisms that include alterations in gene expression (19). Unfortunately, however, the elucidation of the pathways involved in the harmful components of β-adrenergic signaling has been hindered by a lack of a reliable experimental model.

At the cellular level, myocardial failure is characterized by changes in the expression of genes involved in many critical functions of the cardiac myocyte, including those comprising the contractile apparatus. A subset of these molecular changes has been described as a recapitulation of a “fetal” gene program because many embryonically expressed genes that are downregulated postnatally are reactivated, whereas several “adult” genes are repressed (15). Of the changes that are observed in failing hearts, increases in expression of the fetal genes skeletal α-actin and atrial natriuretic peptide (ANP) with coordinate decreases in expression of α-myosin heavy chain (α-MyHC), the ratio of α-MyHC to β-MyHC, and sarcoplasmic reticular ATPase 2a (SRCA2a) are the most widely recognized.

In the intact heart, chronic β-adrenergic stimulation of the myocardium has been shown to change gene expression patterns in a manner that also mimics the fetal gene program observed in failing hearts (3, 19, 27). Several β-AR blocking agents have been identified that successfully treat heart failure patients with DCMs by acting to favorably alter the biology of the failing heart (7). It was recently shown that patients with DCMs who respond to β₁-adrenergic receptor blockade by an improvement in systolic function and a reversal of remodeling also have a reversal of the fetal gene expression (19). In these responder’s hearts, the ratio of α-MyHC to β-MyHC expression and total SRCA2a are increased, whereas atrial natriuretic peptide (ANP) gene expression is reduced (19). Given the evidence of the importance of β-adrenergic signaling in heart muscle disease and heart failure, elucidation of the molecular details of how β-adrenergic stimulation leads to fetal gene induction could lead to the identification of additional therapeutic targets.

Neonatal rat ventricular myocytes (NRVMs) have been used successfully by many investigators as a cell culture model for investigating several signaling pathways involved in heart disease. In the case of β-adrenergic signaling, however, NRVMs have been problematic. That is, treatment of NRVMs with isoproterenol, a nonselective β-AR agonist, induces myocyte hypertrophy similar to that observed in cardiac disease. However, this hypertrophic response does not consistently induce a fetal gene program (18, 31). As a result, little progress has been made in dissecting the effects of β-adrenergic activation. Moreover, the question of whether β-adrenergic stimulation can directly elicit a comprehensive fetal gene response has been raised.

In the work presented here, we demonstrate that the stimulation of NRVMs with 10 nM to 1 μM isoproterenol can consistently induce an upregulation of ANP, brain natriuretic peptide (BNP), and skeletal α-actin expression and a down-
regulation of α-MyHC and SERCA2a. This change is easily monitored by measuring the relative activities of the human and rat cardiac MyHC gene promoters, making this system a valuable model to experimentally examine the molecular pathways involved in β-adrenergic activation. As a proof of principle, we go on to show that the isoproterenol-induced fetal response is specifically mediated by the β1-AR and not the β2-AR. Surprisingly, the β1-AR fetal gene response is independent of cAMP, because treatment of the cells with cAMP analogs had no effect on the activity of MyHC promoters or endogenous fetal and/or adult gene expression, and pretreatment of myocytes with protein kinase A (PKA) inhibitors did not affect isoproterenol-mediated responses. Furthermore, we provide evidence that Ca2+/calmodulin kinase (CaMK) appears to play an important role in the isoproterenol-mediated fetal gene response because inhibition of CaMK resulted in an inhibition of isoproterenol-mediated effects. Finally, we show that isoproterenol-mediated induction of the FGF is dependent on activation of Ca2+ channels because inhibition of the L-type Ca2+ channel (LTCC) channels results in inhibition of the isoproterenol effects on the FGF. Our results suggest that changes in gene expression mediated by β1-adrenergic stimulation occur through a cAMP independent pathway and are likely to occur through a Ca2+-related mechanism.

MATERIALS AND METHODS

Materials. Isoproterenol (Sigma) was dissolved in 1 mmol/l ascorbic acid solution, and the concentrations used are described in RESULTS. Additional reagents were added to the cells with the following final concentrations: 1 μM 8-bromoadenosine 3′,5′-cyclic monophosphate sodium salt (Sigma), 1 μM N7-acetyl-N8-2′-O-dibutyryladenosine 3′,5′-cyclic monophosphate sodium salt (Sigma), 10 μM H-89 (Sigma), 1 μg/ml 14–22 amide myristoylated (Calbiochem), 100 nM prazosin (Sigma), 200 nM CGP-20712A (Tocris), 300 nM ICI-118551 (Tocris), 2 μM KN-93 (Sigma), and 1 μM nicardipine HCl (Sigma). Except as described in the figures, cells were treated with 100 nM isoproterenol 48 h after being plated and harvested 48 h later.

Plasmid construction and preparation. The human α-MyHC promoter construct and rat α-MyHC and β-MyHC promoter construct have been described previously (21, 32). The human ANP promoter (−2593/+18-luc) was a gift of Dr. David Gardner. Plasmid DNA was prepared using alkaline-lysis kits from Qiagen. DNA was eluted in 10 mM Tris and stored at 4°C.

Cell culture and transfections. NRVMs were cultured as previously described (18, 29, 30, 36). Briefly, cells were isolated by trypsin digestion from the ventricles of 1- to 3-day-old rats. Isolation procedures were done in a very gentle manner, and cells were plated at a concentration of 1.5 × 10^5 cells per well in 12-well tissue culture gelatin-coated plates in medium containing 5% bovine calf serum. After 24 h, the medium was changed to serum-free medium containing insulin, transferrin, bovine serum albumin, vitamin B12, and penicillin. Supplements are prepared monthly. Wells were precoated with a 0.1% gelatin solution and dried in a hood under UV lights. In addition, all medium solutions were buffered with HEPES (pH 7.5) to a final concentration of 20 mM. Protocol for animal work is in accordance with PHS Animal Welfare Assurance, ID A3269–01 and approved by the Institutional Animal Care and Use Committee.

Cells were transfected using Fugene 6 (Roche) with 0.25 μg DNA per well. DNA was mixed with Fugene reagent at a ratio of 3 μl Fugene to 1 μg DNA in serum-free medium. Isoproterenol and pharmacological inhibitors were added to the culture medium on the day following transfection. Transfection experiments were done in triplicates in six or more different experiments (n > 18). In cotransfection experiments, the total DNA amount was kept constant by the addition of an empty vector.

RNase protection assay. Total cellular RNA was extracted with TRizol (Invitrogen) for use in RNase protection assays (RPAs). RPAs were performed essentially as described (12, 24). Briefly, 5 μg of RNA were hybridized against [32P]RNA probes specific to skeletal α-actin, SRCA2A, α-MyHC, β-MyHC, ANF, BNP, and GAPDH. RPAs were performed using the RPAII kit (Ambion) from mRNA extracted from a minimum of three different experiments.

Incorporation of [3H]leucine. General cell growth was measured by the incorporation of [3H]leucine as described previously (20). Immediately after treatment of cells with isoproterenol and/or adrenergic receptor blockers, 1 μCi of [3H]leucine was added to each well (12-well plates) of NRVMs. After 48 h, plates were washed twice with ice-cold PBS. The cells were precipitated on ice with 5% trichloroacetic acid. Proteins were then solubilized by the addition of 0.1% SDS and incubation at 37°C on a rocker for 4 h. [3H]leucine incorporation was measured by scintillation counter.

Immunofluorescence. Immunofluorescence was done according to Harrison et al. (9). Cells were washed with Tris buffered saline and 0.1% Tween-20 (TBST) and fixed with 10% formaldehyde for 20 min. Cells were again washed with TBST and incubated with 0.1% Triton-X for 30 min. Cells were then blocked with 1% BSA in TBST for 1 h followed by 1 h incubation with 1:500 dilution of the α-actinin antibody (Sigma A-7811). Cells were then washed with TBST and incubated with 1:1,000 dilution of Alexa 594 anti-mouse antibody (Molecular Probes A11032) for 1 h. Cells were washed three times with TBST and one time with water and sequentially covered with mounting solution (Southern Biotech) and glass coverslips. Images were captured at a ×20 magnification with a fluorescence microscope (Nikon E800) equipped with a digital camera (Zeiss AxioCam) and Zeiss Axiovision version 3.0.6.36 imaging software.

Statistical analysis. All values are means ± SE. All analyses were performed using ANOVA with a P < 0.05 in a two-tailed distribution considered to be statistically significant. The posttest comparison was performed by the method of Bonferroni.

RESULTS

Isoproterenol induces fetal gene expression of pathological markers. Although NRVMs have been used successfully to study the molecular and cellular effects of many pathological-associated biological substances, their use for the examination of β-adrenergic stimulation has yielded conflicting results, making it difficult to study β-AR-mediated signaling pathways in a cell culture model. It is possible that the lack of β-AR response in NRVMs is related to cell density and/or hypertrophic conditions in the media. With this in mind, special care was taken during the isolation and culturing of NRVMs to prevent cell damage, reduce fibroblast contamination, minimize contact-mediated stimulation by plating the cells at low density, and remove all traces of serum after plating.

As shown in Fig. 1, we are able to consistently prepare cells that have measurable pathological responses to β-AR stimulation. Isoproterenol treatment (100 nM) resulted in a decrease in α-MyHC and SRCA2a gene expression, and although β-MyHC gene expression was not increased, there was a decrease in the ratio of α-MyHC to β-MyHC mRNA, which is consistent with a pathological response. Consistent with a pathological response, skeletal α-actin, BNP, and ANP mRNA expression were upregulated (Fig. 1A). Furthermore, isoproterenol treatment causes significant increase in protein synthesis as measured by an increase in the incorporation of [3H]leucine compared with untreated control (Fig. 1B). Measurement of labeled amino acid incorporation has been previously de-
scribed and validated as a marker of cardiac myocyte hypertrophy (16, 17, 29, 30). These data clearly indicate that a fetal gene response is elicited in these cells and confirm our ability to use NRVMs as a model for studying β-AR signaling.

Promoter studies demonstrate fetal response to isoproterenol treatment. To further demonstrate that isoproterenol elicits fetal changes in gene expression in NRVMs and provide a useful technique for measuring these changes, we transfected NRVMs with heterologous promoter constructs in which either the human or rat α-MyHC promoters and the human ANP or rat β-MyHC promoters were driving the expression of luciferase. As shown in Fig. 2, A–D, treatment of NRVMs with 10 nM to 1 μM of isoproterenol results in a fetal response in each of these reporter constructs. More specifically, the human and rat α-MyHC-luc promoter activities are repressed by isoproterenol treatment, whereas the activities of the human ANP-luc and rat β-MyHC-luc reporter constructs are increased. Because the β-AR response elicits repression of α-MyHC promoter at a 10 nM to 1 μM isoproterenol range, all subsequent experiments were performed with 100 nM isoproterenol.

Fetal gene response is mediated through β1-AR and not through β2-AR. To confirm that the fetal gene response observed in the promoter-reporter experiments is specifically mediated through β-ARs, the pharmacological β-AR antagonist propranolol was used in combination with isoproterenol treatment of NRVMs. As shown in Fig. 3, the addition of propranolol prevented the isoproterenol-mediated repression of both the human and rat α-MyHC-luc reporters as well as the activation of the β-MyHC-luc reporter.

These data led us to ask which β-adrenergic receptor, β1-AR or β2-AR, is mediating the fetal gene response. To answer this question, each receptor was blocked individually by the addition of the receptor-specific blockers CGP-20712A and ICI-118551, which selectively inhibit the β1-AR and β2-AR, respectively. As shown in Fig. 3, CGP-20712A, but not ICI-118551, blocks the isoproterenol-mediated changes in promoter activities of our reporter constructs, indicating that the fetal gene response to isoproterenol treatment is mediated through β1-ARs and not through β2-ARs.

Endogenous changes are specifically mediated by β1-AR. To confirm that our reporter studies were accurately reflecting the regulation of endogenous responses to isoproterenol through the β1-AR, mRNA levels of the fetal gene program were measured. As shown in Fig. 4A, repression of SRCA2a and α-MyHC, and activation of BNP, ANP, and skeletal α-actin in response to isoproterenol are all blocked by CGP-20712A, but
not by ICI-118551, consistent with the promoter-reporter system. Increase in protein synthesis, however, was blocked by the β1-AR blocker, CGP-20712A and by the β2-AR blocker ICI-118551 (Fig. 4B). Taken together, these data demonstrate that isoproterenol induces a pathological response in NRVMs that is specifically mediated by activation of the β1-adrenergic receptor.

Isoproterenol-mediated changes in the fetal gene program do not occur through α-AR. To rule out the possibility that the changes in promoter activity were not mediated by α1-ARs, cells were treated with 1 μM of the specific α1-AR antagonist prazosin before treatment with isoproterenol. α1-AR blockade did not affect α-MyHC, β-MyHC, or ANP promoter activity at isoproterenol concentrations of 100 nM or 1 μM, providing further evidence that lower concentrations of isoproterenol induces the fetal gene program through a β-adrenergic-mediated pathway (Fig. 5). However, at higher concentrations, prazosin reduced the isoproterenol effect, suggesting that at concentrations higher than 1 μM, isoproterenol activation is leaky and stimulates the α-AR pathway.

Fig. 2. Human and rat α-MyHC promoters (A and C) and human ANP (B) and rat β-MyHC (D) promoters were treated with increasing concentrations of ISO. Luciferase activity was measured 48 h after treatment. N.S. not significant.

Fig. 3. Changes in promoter activity are mediated by β1-adrenergic receptor (β1-AR). NRVMs were treated with 1 μM of the β-AR blocker propranolol 15 min before ISO treatment. Luciferase activity was measured 48 h after ISO treatment. To determine which β-AR is involved in changes in promoter activity, 200 nM of the β1-AR-specific inhibitor CGP-20712a and 300 nM of the β2-AR-specific inhibitor ICI-118551 were added to the cells 15 min before treatment with 100 nM ISO. A and B: MyHC and ANF promoter response to ISO is mediated by β1- and not β2-AR activity. Cells were harvested, and luciferase activity was measured 48 h after ISO treatment.
Inhibition of isoproterenol-activation of the FGP is independent of PKA. In cardiac myocytes, many β₁-adrenergic responses are mediated through cAMP activation of PKA (26). To determine whether PKA is involved in isoproterenol-mediated changes in α-MyHC promoter activity, cells were treated with 8-bromo-cAMP or dibutyryl cAMP. The α-MyHC promoter-reporter system failed to respond to 8-bromo-cAMP (Fig. 6A) or dibutyryl cAMP (data not shown). We then sought to determine whether the small molecule PKA inhibitor H-89, or the PKA peptide inhibitor PKI14–22 was capable of blocking the isoproterenol-mediated effect. Neither PKI14–22 nor H-89 blocked isoproterenol-mediated repression of the human α-MyHC promoter (Fig. 6B), human ANP, and rat β-MyHC promoters (data not shown). PKA inhibition did not prevent the downregulation of α-MyHC, although it did reduce β-MyHC gene expression. PKA inhibition failed to prevent downregulation of SRCA2A. Although PKA inhibition reduced the degree of inhibition of ANP, BNP, and skeletal α-actin, these genes were still upregulated in response to isoproterenol (Fig. 8).

To confirm the results of the pharmacological treatment, cells were transfected with the human α-MyHC-luc vector promoter and a construct that expresses a constitutively active form of PKA (caPKA). caPKA did not change the activity of the α-MyHC promoter (Fig. 6C). As a positive control for PKA-mediated effects, a synthetic promoter construct containing three copies of the cAMP response element binding site was cotransfected with caPKA in NRVMs; this construct was upregulated by the caPKA (data not shown).

Isoproterenol-mediated changes in promoter activity and mRNA expression are regulated through CaMKII. In cardiac myocytes it was recently shown that CaMKII is involved in the pathological aspect of the β₁-AR-mediated response (35, 37, 40). It has also been shown that β-adrenergic stimulation induces expression of cardiac ankyrin repeat protein expression in NRVMs through a CaMK- and PKA-dependent pathway (41). Moreover, CaMK has been implicated in the regulation of the hypertrophic markers skeletal α-actin, BNP, ANF, and β-MyHC (see Refs. 22 and 39 and review in Ref. 38). We next...
sought to determine whether CaMKII plays a role in the changes in gene expression mediated by β1-AR in cardiac myocytes. Inhibition of CaMKII by KN-93 prevented the majority of endogenous mRNA fetal gene program induction (Fig. 7A). KN-93 pretreatment prevented isoproterenol-associated downregulation of α-MyHC or upregulation of skeletal α-actin and natriuretic peptides. However, inhibition of CaMK was not capable of preventing repression of SRCA2a, suggesting that SRCA2a gene repression is regulated by a different pathway. Surprisingly, KN-93 alone resulted in a decrease in ANP, β-MyHC, and SRCA2a mRNA levels. These results suggest that inhibition of CaMKII not only blocks the effect of isoproterenol but results in repression of certain genes that are upregulated in NRVMs under the cell culture conditions used in this study.

Isoproterenol-activation of FGP is mediated by LTCC. The results presented above suggest that Ca2+ is an important mediator of fetal gene activation in response to β1-AR stimulation. β1-AR signaling has been shown to activate LTCCs through Gαs (14), and CaMKII has been shown to activate LTCCs (1), both independently of cAMP activation, making LTCCs a likely candidate for Ca2+ regulation in response to β1-AR activation. To determine whether LTCC participates in the isoproterenol-mediated activation of the fetal gene program, NRVMs were pretreated with the LTCC inhibitor nicardipine. As shown in Fig. 8, inhibition of LTCCs with nicardipine prevented downregulation of α-MyHC and SRCA2a and upregulation of ANP, BNP, and skeletal α-actin. Interestingly, inhibition of LTCC increased upregulation of β-MyHC in response to isoproterenol stimulation.

DISCUSSION

In this study we demonstrate that isoproterenol induces a hypertrophic response in NRVMs as measured by an induction of the fetal gene program as well as an increase in cellular incorporation of radiolabeled leucine. The changes in gene expression invoked by isoproterenol were similar to those observed in the intact hearts of adult animals and human subjects exhibiting pathological hypertrophy and myocardial failure (3, 19). This fetal gene expression response to isoproterenol is β1-AR specific and is not mediated through β1-ARs. The fetal gene response is mediated through a β1-AR receptor pathway that is cAMP and PKA independent but dependent on CaMKII and LTCC.

Previous studies in NRVMs were unable to detect a change in endogenous MyHC levels with β1-adrenergic stimulation, suggesting that in this system β-adrenergic pathways were not involved in the fetal gene response (31), despite the fact that β-adrenergic stimulation has been shown to increase ANP gene expression (2, 23). This lack of a comprehensive β-adrenergic fetal gene induction in NRVMs was in contrast to results in the adult rat intact myocardium as well as clinical data in the human heart (3, 19, 27). However, our data indicate that
NRVM treatment with isoproterenol demonstrated a fetal gene response as assessed by the activities of $\alpha$-MyHC, $\beta$-MyHC, and ANP reporter constructs, as well as an increase in protein synthesis based on increased levels of $[^3]$H]leucine incorporation. As argued previously (18), the hypertrophy observed in NRVMs upon treatment with isoproterenol could be dependent on increased contractility and thus might not be associated with the changes in gene expression that ordinarily accompany pathological hypertrophy. However, we demonstrated that $\beta$-adrenergic stimulation alters the activities of the human and rat cardiac MyHC promoters and human ANP promoter in directions consistent with the “fetal” gene response that is considered to be the molecular marker of pathological hypertrophy (33). Several important conclusions can be drawn from these data. First, the use of transiently transfected luciferase reporters can serve as a convenient assay for measuring the pathological effects of isoproterenol treatment on NRVMs. The results of the luciferase assays closely mimicked the

![Graphs and images showing data](https://example.com/graphs.png)
endogenous mRNA data shown in Fig. 1, providing a useful tool for observing the fetal gene response in these cells. Second, the ability of isoproterenol to elicit a pathological response of the \( \beta_1 \)-MyHC promoter falls within a defined range of isoproterenol concentrations. As can be seen in Fig. 2, A and C, there is no significant response of the \( \alpha \)-MyHC promoter when the isoproterenol concentration is lower than 10 nM or higher than 1 \( \mu \)M. At lower concentrations, there is likely insufficient activation of the \( \beta \)-ARs to elicit a fetal response. At higher concentrations, however, the \( \alpha \)-AR pathway is activated that eclipse \( \beta \)-adrenergic signaling, preventing the repression of the \( \alpha \)-MyHC promoter.

Moreover, isoproterenol induced a fetal pattern of endogenous gene expression in NRVMs with repression of SRCA2a mRNA expression and activation of skeletal \( \alpha \)-actin, BNP, and ANP mRNA expression. Importantly, the \( \alpha \)-MyHC-to-\( \beta \)-MyHC ratio is downregulated, and the alteration in this balance is likely responsible for the changes in contractile activity as seen in experiments with single cells (10). Changes in \( \alpha \)-MyHC-to-\( \beta \)-MyHC ratios without activation of \( \beta \)-MyHC gene expression have also been observed in failing human hearts (19). Taken together, these data demonstrate that \( \beta \)-adrenergic pathway is able to induce "pathological" hypertrophy and a fetal gene program response in NRVMs.

Inhibition of \( \beta_1 \)-AR resulted in complete blockade of isoproterenol-mediated repression or activation of the promoters analyzed or endogenous gene expression, whereas \( \beta_2 \)-AR blockade did not change the isoproterenol-mediated effects. This corroborates previous work in NRVMs demonstrating that isoproterenol-mediated increases in ANP gene transcription and hypertrophy are \( \beta_1 \)-AR dependent, and data showing that the use of \( \beta_1 \)-adrenergic blockers can reverse fetal gene expression patterns in vivo (19, 23). Increase in protein synthesis was repressed by both \( \beta_1 \)-AR and \( \beta_2 \)-AR blockers (Fig. 4, A

Fig. 7. Activation of \( \beta_1 \)-AR-mediated fetal gene expression is dependent on Ca\(^{2+}/\)calmodulin-dependent protein (CaMKII). NRVMs were pretreated with KN-93 for 1 h followed by ISO treatment as described in the text. RNA was extracted and analyzed by RPA. Bars represent a total of three individual experiments. Each mRNA was normalized to GAPDH.

Fig. 8. L-type Ca\(^{2+} \) channels (LTCCs) are involved in \( \beta_1 \)-AR mediated fetal gene expression. NRVMs were pretreated with nicardipine for 15 min followed by ISO treatment as described in the text. RNA was extracted and analyzed by RPA. Bars represent a total of three individual experiments. Each mRNA was normalized to GAPDH.
Stimulation of β-AR by catecholamines results in the activation of the β-AR-adenylyl cyclase-PKA cascade (34), classically thought to mediate most of the biological responses to β-adrenergic stimulation. For this reason, it was surprising that PKA inhibition by different methods was incapable of blocking isoproterenol-mediated effects on any aspect of fetal gene induction, although it reduced upregulation of ANP, BNP, and skeletal α-actin. These results suggest that cAMP-PKA pathway might be involved in a minor upregulation of the fetal gene program, but it is not responsible for the changes observed in response to isoproterenol treatment. These findings are in agreement with Bogoyevitch et al. (2), who demonstrated in NRVMs that isoproterenol-mediated hypertrophy and increased ANP promoter activity utilized cAMP independent signaling.

Recent work in NRVMs has shown that CaMKII is involved in β-AR-mediated response in both cases independent of cAMP and PKA (35, 37, 40). Here we show that the pathological changes in gene expression in response to β1-AR stimulation are also dependent on activation of CaMKII, with the exception of SRCA2a. SRCA2a repression was not blocked by CaMKII inhibition suggesting that regulation of SRCA2a gene expression is mediated by a different pathway. Finally, activation of CaMKII is dependent on cellular Ca2+ influx, so we next determined the role of Ca2+ channels in β1-AR response. Inhibition of LTCC resulted in blockade of β1-AR activation of fetal gene induction, suggesting that Ca2+ plays a major role in initiation of this response.

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DISCLOSURES
M. R. Bristow is an Officer and has equity in Myogen Inc. and ARCA Discovery, Inc.; L. A. Leinwand has equity in Myogen, Inc.

REFERENCES


