GATA4 is a survival factor in adult cardiac myocytes but is not required for α1A-adrenergic receptor survival signaling

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1A-adrenergic receptor survival signaling in adult cardiac myocytes. We measured norepinephrine (NE)-induced cell death in cultured cardiac myocytes lacking α1-ARs (cultured from α1B/AR double-knockout mice, α1BKO mice) that are susceptible to cell death induced by several proapoptotic stimuli, including NE. Our results show that overexpression of GATA4 is sufficient to protect α1BKO cardiac myocytes from NE-induced cell death. However, we found that the α1A-subtype did not induce phosphorylation or increase the activity of GATA4 in adult mouse cardiac myocytes in culture or in vivo. Furthermore, we examined the effect of siRNA-mediated knockdown of GATA4 on α1A-survival signaling. In α1B-knockout cardiac myocytes, which express only the α1A-subtype and are protected from NE-induced cell death, GATA4 knockdown did not reverse α1A-survival signaling in response to NE. In summary, we found that GATA4 acted as a survival factor by preventing cell death in α1ABKO cardiac myocytes, but GATA4 was not activated by α1-AR stimulation and was not required for α1A-survival signaling in adult cardiac myocytes. This also identifies an important mechanistic difference in α1-signaling between adult and neonatal cardiac myocytes.

α1-ARs are classically associated with regulating vascular smooth muscle cell contractility and blood pressure (3). However, recent studies indicate that α1-ARs also mediate many important functions in the heart, including postnatal hypotrophy, contractile function, and survival signaling (10, 14, 17, 19). In mice with systemic deletion of the α1A- and α1B-AR subtypes (α1ABKO mice), which lack α1-ARs in cardiac myocytes, we recently demonstrated that α1-ARs are required for adaptation to pathologic pressure overload (19). Specifically, aortic constriction induced apoptosis, dilated cardiomyopathy, and death in α1ABKO mice (19). Subsequently, we found that α1ABKO myocytes were susceptible to cell death induced by several pro-death agonists, including NE (10, 19). By reconstituting α1A-subtype signaling in α1ABKO myocytes, we defined an α1A-AR extracellular-signal regulated kinase (α1A-ERK) signaling pathway that mediates survival signaling in adult cardiac myocytes (10). The absence of this α1A-ERK signaling pathway in α1ABKO cardiac myocytes could explain the maladaptive response following aortic constriction (10). In summary, our previous studies define a novel protective function of cardiac myocyte α1-ARs to prevent myocyte death (survival signaling) and attenuate ventricular remodeling in response to pathologic stress, which agrees with clinical studies examining α1-blockade in heart disease.

In this study, we sought to identify mechanisms mediating α1A-survival signaling downstream of ERK activation in cardiac myocytes. Previous reports suggest that α1-survival signaling could be mediated by several different mechanisms, including the phosphorylation and inactivation of the Bcl-2 family member Bad downstream of ERK (24), the activation of nuclear factor of activated T-cells (NFAT) signaling downstream of calcineurin (21), or by activation of the cardiac-specific transcription factor GATA4 downstream of ERK. Of these potential signaling pathways, ERK-mediated activation of GATA4- and GATA4-mediated survival signaling are the best characterized pathways in cardiac myocytes. Previous studies in cultured neonatal rat cardiac myocytes indicate that GATA4 is directly phosphorylated by ERK following α1-AR activation, which increases GATA4 DNA binding and transcriptional activity (13, 15). GATA4 also acts as a survival factor by increasing Bcl-2 and Bcl-XL expression and preventing doxorubicin-induced apoptosis in neonatal rat cardiac myocytes (2, 11).

Therefore, we examined the cardiac-specific transcription factor GATA4 as a potential downstream effector of α1A-ERK...
survival signaling in adult mouse cardiac myocytes. Here, we report that GATA4 acted as a survival factor by preventing cell death in adult mouse cardiac myocytes lacking α1-ARs. However, contrary to prior studies in neonatal rat cardiac myocytes, we found that GATA4 was not activated by α1-ARs and was not required for α1A-survival signaling in adult mouse cardiac myocytes. These findings identify an important mechanistic difference in α1-AR function between adult and neonatal cardiac myocytes.

**METHODS**

**Adenoviral constructs.** Adenoviruses expressing α1A-green fluorescent protein (GFP), GATA4, and a constitutively active MEK1 mutant were generated, as described previously (4, 10, 12). Briefly, to generate the α1A-GFP fusion protein, the cDNA for the human α1A-AR (NM000680) was amplified by PCR with primers designed to remove the stop codon and insert Bgl II and Mlu I restriction sites at the 5’ and 3’ ends, respectively. The amplified α1A product was cloned into pCR2.1-TOPO (Invitrogen, Carlsbad, CA), then subcloned into the Bgl II-Mlu I restriction sites in the multicloning site of the humanized pGFP-2-N3 vector (BioSignal Packard, Montreal, Quebec, Canada), with GFP at the C-terminus (5, 16, 20, 23).

To generate adenovirus expressing the α1A-GFP fusion protein under control of the cytomegalovirus (CMV) promoter, the α1A-AR-GFP was amplified by PCR with primers designed to insert Pme I and Xba I restriction sites at the 5’ and 3’ ends, respectively. The amplified α1A-GFP product was cloned into the pCR2.1-TOPO vector (Invitrogen, Carlsbad, CA), then subcloned into the pBgl II-Xba I restriction sites in the Ad5CMV K-Neo vector (ViraQuest, North Liberty, IA) under control of the CMV promoter. The Ad5-plasmid with the α1A-AR-GFP insert was then recombined with an adenoviral cell line. Clones positive for recombination were transfected into HEK293 cells. Viral products evident 7–10 days after transfection were amplified, purified through two rounds of CsCl gradients, and dialyzed against a 3% sucrose/PBS solution. Viral titer was determined by observing plaque formation in agarose overlay assays. Expansion and purification of adenoviruses expressing GATA4 or the constitutively active MEK1 mutant followed the same procedures.

**Mice.** The α1-AR knockout mice used in this study were previously described (17, 19). In all experiments, we used congenic male wild-type (WT) or knockout (KO) mice, aged 10–15 wk. All protocols involving animal use were approved by the Internal Animal Care and Use Committee at The University of South Dakota. This investigation conforms to the Guide for the Care and Use of Laboratory Animals published by the U.S. National Institutes of Health (NIH Publication No. 85-23, revised 1996).

**Culture of adult mouse cardiac myocytes, adenoviral infection, and transfection of siRNA oligonucleotides.** Ventricular cardiac myocytes from adult male mice were cultured as previously described (10, 18, 19). Briefly, hearts were removed, cannulated, and perfused with collagenase type II (Worthington Biochemical, Lakewood, NJ) to dissociate ventricular myocytes. Cardiac myocytes were plated at a density of 50 rod-shaped myocytes per square millimeter on laminin-coated 35-mm culture dishes. Cardiac myocytes were cultured in MEM with Hank’s balanced salt solution, 1 mg/ml bovine serum albumin, 10 mM 2,3-butanedione monoxime, and 100 U/ml penicillin in a 2% CO₂ incubator at 37°C.

Cardiac myocytes were infected with adenovirus by adding virus directly to the culture medium following plating. Cardiac myocytes were transfected with siRNA oligonucleotides (300 nM) directed against GATA4, GAPDH, or a scrambled oligonucleotide following plating using Lipofectamine RNAiMAX (Invitrogen, Carlsbad, CA) following manufacturer’s instructions with one exception. We did not use Opti-MEM, as suggested, because of toxicity in our culture system, but substituted our culture medium (see above). The GATA4 siRNA oligonucleotide sequence was 5’-GGAGGGGAUCAAAC-CAGAAT-3’, and GAPDH and scrambled oligonucleotides were purified from Ambion (Austin, TX). RNA levels were measured by RT-PCR, as previously described (11).

**Cultivation of neonatal rat cardiac myocytes.** Neonatal rat cardiac myocytes were cultured following established procedures originally described by Simpson (22).

**Measurement of cell death.** Myocyte death was measured using annexin V/propidium iodide staining, as described previously (10, 19). For cell death assays, cardiac myocytes were infected with adenovirus or transfected with siRNA oligonucleotides and cultured for 40 h. At 40 h, cardiac myocytes were treated for 2 h with L-norepinephrine bitartrate (NE, 1 μmol/l) or vehicle (100 μmol/l ascorbic acid). After 2 h, annexin V-Fluos (AnnV, Roche Diagnostics, Indianapolis, IN) and propidium iodide (PI, Roche Diagnostics) were added to the culture medium. After 10 min, myocytes were photographed under both phase contrast and fluorescent microscopy. For each condition, 300–400 myocytes were counted in randomly selected fields, and each condition was measured in duplicate. Apoptotic myocytes were defined as AnnV positive and PI negative, and necrotic myocytes were defined as AnnV and PI positive. Total cell death was defined as the sum of apoptotic and necrotic cells.

**Western blot analysis.** Protein extracts from cultured adult mouse cardiac myocytes or heart tissue were prepared, as described previously (4, 10, 19). Phospho-GATA4, total GATA4, Bcl-2 levels, phospho-ERK, and total ERK levels were all detected by Western blot analysis (antibody to phospho-GATA4 from BioSource, Camarillo, CA; antibodies to total-GATA4, Bcl-2 from Santa Cruz Biotechnology, Santa Cruz, CA; antibodies to phospho- and total-ERK from Cell Signaling Technology, Beverly, MA; and antibodies to glyceraldehyde-3-phosphate dehydrogenase, GAPDH, from Research Diagnostic, Flanders, NJ).

**Transverse aortic constriction.** Surgery was performed without intubation under anesthesia with isoflurane, as previously described (17, 19).

**ERK immunocytochemistry.** WT adult mouse and neonatal rat cardiac myocytes were cultured on laminin-coated glass coverslips. For immunocytochemistry, cardiac myocytes were blocked for 1 h before the addition of primary antibody directed against phosphorylated ERK (Cell Signaling Technology, Beverly, MA) and were incubated with conjugated secondary antibodies (Texas red anti mouse, Invitrogen, Carlsbad, CA) for 1 h before mounting with Fluoromount G. Fluorescent images were captured by confocal microscopy using Flouview software (Olympus BX50 confocal microscope; Olympus America, Melville, NY). Images were processed for publication using Imaris software (Bitplane Scientific Solutions, St. Paul, MN).

**Statistics.** In all experiments, values were compared by Student’s t-test or one-way ANOVA with means compared by Tukey post-test, and P < 0.05 was considered significant. The number of experiments (n), given in each figure legend, refers to independent cultures from different hearts.

**RESULTS**

**GATA4 is sufficient to protect α1ABKO cardiac myocytes from NE-induced cell death.** Previously, we demonstrated that α1ABKO cardiac myocytes were susceptible to cell death by numerous cell death stimuli, including NE, H₂O₂, isoproteonor, and doxorubicin, and defined an α1A-ERK survival signaling pathway in adult mouse cardiac myocytes (10, 19). To identify downstream effectors of this α1A-ERK survival signaling pathway, we examined the cardiac-specific transcription/survival factor GATA4, which is downstream of α1-ERK signaling in neonatal cardiac myocytes (12, 13). If GATA4 mediates α1A-survival signaling, the absence of α1A-ERK

**FIGURE 2.** Western blot analysis of phospho-ERK, total-ERK, and GATA4 in control (WT) and α1A-ERK mutant (α1AABKO) adult cardiac myocytes. Full-sized blot is provided as Supplemental Figure 2.
Fig. 1. GATA4 is sufficient to protect α1ABKO cardiac myocytes from norepinephrine (NE)-induced cell death. A: annexin V assay in cultured α1ABKO cardiac myocytes expressing the α1A-GFP or GATA4. α1ABKO cardiac myocytes were infected with adenovirus encoding α1A-GFP [multiplicity of infection (MOI) 1,000], GATA4 (MOI 100), or β-galactosidase (βgal, MOI 1,000) and cultured for 40 h. At 40 h, cardiac myocytes were treated for 2 h with 1 μmol/l NE or vehicle (100 μmol/l ascorbic acid), and cell death was assayed using Annexin-V/propidium iodide staining. Cardiac myocytes were photographed under phase contrast (left) and fluorescence (right) to determine the percent of apoptotic cells (AnnV positive/PI negative) and necrotic cells (AnnV/PI positive). Magnification, 100×. Cell death (B) and myocyte morphology (C) are shown. The percent of apoptotic/necrotic cardiac myocytes (B) and rod-shaped/round cardiac myocytes (C) were calculated from 300–400 myocytes per condition (n = 4–6 independent cultures). Total cell death (B) (apoptosis plus necrosis) and rod-shaped morphology (C) in each group were compared by one-way ANOVA with Tukey post-test. P < 0.05 for all groups for both cell death and morphology, and significant differences between groups are shown. D: overexpression of GATA4 in cultured α1ABKO cardiac myocytes. α1ABKO cardiac myocytes were infected with adenovirus encoding GATA4 or βgal as in A. After 40 h, whole cell homogenates were prepared and GATA4 levels were measured by Western blot analysis. *MOI used in A–C. Because of the high level of expression of GATA4 in cardiac myocytes infected at 1,000 MOI, GATA4 in the βgal-infected cardiac myocytes is not visible on the blot at this exposure.
signaling and failure to phosphorylate and activate GATA4 in α1ABKO cardiac myocytes might explain their susceptibility to cell death.

Initially, we tested whether overexpression of GATA4 could rescue α1ABKO cardiac myocytes from NE-induced cell death. NE, which is increased in response to pathologic stress in the heart, induces cardiac myocyte death through activation of β-ARs (9, 26). In these experiments, α1ABKO cardiac myocytes were infected with adenoviruses encoding GATA4, an α1A-AR-GFP fluorescent fusion protein (α1A-GFP) (10), or β-galactosidase (control). Cardiac myocyte death was induced by NE (1 μmol/l) and measured by AnnV/PI staining.

Fig. 2. siRNA-mediated knockdown of GATA4 does not reverse α1A-mediated survival signaling in α1BKO cardiac myocytes. A, B: siRNA mediated knockdown of GATA4. A: Wild-type (WT) cardiac myocytes were transfected with siRNA oligonucleotides directed against GAPDH or GATA4 (300 nmol/l), and after 40 h, RNA levels for both were measured by RT-PCR. B: WT cardiac myocytes were transfected with siRNA oligonucleotides directed against GATA4 (or a scrambled oligonucleotide control, siCon), and after 40 h, GATA4 protein levels were measured by Western blot analysis and knockdown was quantified by densitometry (standardized to GAPDH). Groups were compared by Student’s t-test (n = 4). C, D: Annexin V Assay in cultured α1BKO cardiac myocytes transfected with siRNA against GATA4 (siGATA4) or a scrambled oligonucleotides (siCon) (300 nmol/l). α1ABKO cardiac myocytes treated with NE were included for comparison. At 40 h, cardiac myocytes were treated with NE and Annexin V assays were performed as in Fig. 1. Total cell death (C) and rod-shaped morphology (D) in each group were compared by one-way ANOVA with Tukey post-test. P < 0.05 for all groups for cell death and morphology, and significant differences between groups are shown.

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NE increased cell death in α1ABKO cardiac myocytes (cell death, apoptosis, and necrosis: βgal, 8.2 ± 1.7%; βgal NE, 21.4 ± 2.5%, \( P < 0.05 \)) and reconstitution of α1A-subtype signaling prevented NE-induced cell death (cell death: α1A, 8.8 ± 3.9%; α1A NE, 9.4 ± 4% \( P < 0.05 \) vs. βgal NE), (Fig. 1, A–C). These results replicated our previous finding that NE increased cell death in α1ABKO cardiac myocytes, relative to WT cardiac myocytes and that reconstitution of α1A-signaling completely reversed the susceptibility of α1ABKO cardiac myocytes to cell death (10, 19). More importantly, overexpression of GATA4 (Fig. 1D) protected α1ABKO cardiac myocytes from NE-induced cell death (cell death: GATA4, 10.2 ± 1.1%; GATA4 NE 13.9 ± 1.3% \( P < 0.05 \) vs. βgal NE) (Fig. 1, A–C). In summary, GATA4 is sufficient to protect α1ABKO myocytes from NE-induced cell death. siRNA-mediated knockdown of GATA4 does not reverse α1A-mediated survival signaling in α1BKO cardiac myocytes.

To test directly whether GATA4 is required for α1A-survival signaling, we examined the effect of reduced GATA4 levels on α1A-survival signaling by using small interfering RNA (siRNA) targeted against GATA4. To demonstrate the efficacy of the GATA4 siRNA, we transfected WT cardiac myocytes with siRNA oligonucleotides (300 nM) targeted against both GATA4 and GAPDH and measured mRNA levels by RT-PCR. Both GATA4 and GAPDH mRNA levels were specifically reduced by their respective siRNA oligonucleotides (Fig. 2A). In WT cardiac myocytes transfected with GATA4 siRNA oligonucleotides (300 nM) or control scrambled siRNA, we also measured GATA4 protein levels by Western blot analysis. GATA4 protein levels were reduced by 62% (Fig. 2B, \( P < 0.05 \), \( n = 4 \)), which we believe is the first demonstration of siRNA-mediated knockdown in cultured adult mouse cardiac myocytes.

To measure the effect of GATA4 knockdown on α1A-survival signaling, we used α1B-single knockout cardiac myocytes, which express only the α1A-subtype, instead of reconstituting α1A-signaling in α1ABKO cardiac myocytes as before. This was done to avoid toxicity caused by the combi-
nation of the siRNA transfection reagent and adenovirus. Recapitulating our previous results, we found that α1B-single knockout (α1BKO) cardiac myocytes were protected from NE-induced cell death relative to α1ABKO cardiac myocytes [cell death: α1ABKO, 8.2 ± 1.7%; α1ABKO NE, 21.4 ± 2.5%, P < 0.05; α1BKO, 8.8 ± 1.2%; α1BKO NE, 11.6 ± 1.1%, P = nonsignificant (NS) vs. vehicle, but P < 0.05 vs. α1ABKO NE] (Fig. 2, C and D) (10). In this experiment, if GATA4 were required for α1A-survival signaling, then we would predict that GATA4 knockdown should reverse the protective effects of the α1A-subtype in α1BKO cardiac myocytes. However, this was not the case, as NE had no significant effect on cell death (P = NS) in α1B-single-knockout cardiac myocytes transfected with GATA4 siRNA compared with control scrambled siRNA (α1BKO siGATA4, 9.2 ± 1.6%; α1BKO siGATA4 NE, 7.6 ± 0.5%, P = NS vs. α1BKO NE) (Fig. 2, C and D). In summary, these experiments suggested that while GATA4 is sufficient to protect α1ABKO cardiac myocytes from NE-induced cell death, GATA4 is not required for α1A-mediated survival signaling in adult mouse cardiac myocytes.

The α1A-subtype does not induce phosphorylation of GATA4 in adult mouse cardiac myocytes. Previously, we found that the α1A-subtype activates ERK with greater efficacy than the α1B-subtype in adult mouse cardiac myocytes, which correlates with α1A-survival signaling (10). In both cultured neonatal rat cardiac myocytes and neonatal mice in vivo, activation of ERK by α1-AR stimulation induces phosphorylation of GATA4 at S105, which increases GATA4 DNA binding and transcriptional activity (6, 13). To understand why GATA4 was not required for α1A-mediated survival signaling in adult mouse cardiac myocytes, we measured α1A-subtype-mediated phosphorylation of GATA4 using the α1-AR selective agonist phenylephrine (PE) instead of NE, which avoided the complication of NE activation of β-ARs (at the concentration used). Because phosphorylation of GATA4 is required for GATA4 DNA binding activity and transcriptional activity, we used it as a marker of GATA4 activity (13). As expected, PE...
(20 μmol/l) increased the phosphorylation of ERK in both WT cardiac myocytes and α1ABKO cardiac myocytes expressing the α1A-GFP at 0.5 and 3 h, which returned to control levels by 24 h (Fig. 3A, n = 2–4, representative blots shown), similar to our previously published results (10). Interestingly, PE did not increase the phosphorylation of GATA4 in either WT cardiac myocytes or α1ABKO cardiac myocytes expressing the α1A-GFP (Fig. 3A, n = 2–4, representative blots shown). However, positive controls, PMA (100 nM) and a constitutively active MEK1 mutant (upstream activator of ERK), increased the phosphorylation of GATA4 (Fig. 3B, n = 3, representative blot shown) in α1ABKO cardiac myocytes. Further, PE did not increase levels of the survival factor Bcl-2 in either WT cardiac myocytes or α1ABKO cardiac myocytes expressing the α1A-GFP (Fig. 3A, n = 4 at 0.5 h, n = 2 at 3, 24 h, representative blot shown), which was increased by GATA4 activation in neonatal rat cardiac myocytes (11), indicating that GATA4 transcriptional activity was likely not induced by α1-stimulation in adult mouse cardiac myocytes. In contrast, we found that PE increased the phosphorylation of ERK and GATA4, as well as increased the levels of Bcl-2 in neonatal rat cardiac myocytes, as previously reported (Fig. 3C, n = 3, representative blots shown) (11). Therefore, our results identify important differences in α1-GATA4 signaling between adult and neonatal cardiac myocytes and suggest that failure to activate (phosphorylate) GATA4 explains the lack of requirement for GATA4 in α1-mediated survival signaling in adult cardiac myocytes.

**Aortic constriction does not change phosphorylation of GATA4 in α1ABKO mice.** We previously found that aortic constriction, which increases sympathetic nervous system-mediated NE release in the heart, induces dilated cardiomyopathy and death in α1ABKO mice (10, 19). Specifically, aortic constriction caused death in 40% of α1ABKO mice, while the surviving mice developed a severe dilated cardiomyopathy, with a significant twofold reduction in ejection fraction and twofold increase in end-diastolic volume relative to WT (19). At the cellular level, aortic constriction induced a four-fold increase in apoptosis in α1ABKO hearts relative to WT (19). This maladaptive response to aortic constriction correlates with a failure to activate the α1A-ERK survival-signaling pathway. In the absence of α1A-ERK signaling, the increased NE release caused by aortic constriction likely led to unopposed activation of β-ARs, resulting in the maladaptive response observed in α1ABKO mice.

Here, we measured GATA4 phosphorylation following aortic constriction in WT and α1ABKO mice (Fig. 4). Previously, we found that aortic constriction increased the phosphorylation of ERK slightly in WT mice, but in α1ABKO mice, phosphorylation of ERK was decreased in sham-operated animals relative to WT, and even further decreased by aortic constriction (10). However, aortic constriction had no effect on phosphorylation of GATA4, as the ratio of phospho-GATA4 to total GATA4 was unchanged in WT and α1ABKO mice (P = NS). In addition, aortic constriction had no effect on Bcl-2 levels (P = NS). This discordance, in which phospho-GATA4 was not changed in α1ABKO mice relative to WT mice, but phospho-ERK was reduced, would also suggest that GATA4 is not downstream of α1-ERK signaling in adult mouse heart.

**α1-AR-mediated activation of ERK induces localization of phosphorylated-ERK to the plasma membrane in adult cardiac myocytes.** Our results suggested a disconnect between ERK-mediated phosphorylation of GATA4 downstream of α1-ARs in adult cardiac myocytes that was previously observed in neonatal cardiac myocytes. To establish a mechanistic basis for this difference in α1-mediated GATA4 signaling in adult vs. neonatal cardiac myocytes, we examined ERK localization following α1-activation. Interestingly, we found that phospho-ERK localized to the plasma membrane following α1-stimulation in adult cardiac myocytes but was found throughout the cell in neonatal cardiac myocytes (Fig. 5). This difference in ERK localization could explain the failure of α1-mediated phosphorylation of GATA4 and provide a mechanistic expla-
nation for our observation that GATA4 is not required for α1-mediated survival signaling in adult cardiac myocytes.

DISCUSSION

Here, we examined the role of GATA4 in α1A-subtype-mediated survival signaling in adult mouse cardiac myocytes. In adult cardiac myocytes lacking α1-ARs (α1ABKO myocytes), which are susceptible to cell death from multiple stimuli, including NE (10, 19), we found that GATA4 overexpression attenuated NE-induced cell death. The finding that GATA4 is sufficient to protect α1ABKO myocytes from NE-induced cell death agrees with previous reports that demonstrate GATA4 is a survival factor in neonatal cardiac myocytes (2, 11). Although GATA4 was sufficient to protect α1ABKO myocytes, GATA4 was not required for α1A-subtype-mediated survival signaling. We observed that siRNA-mediated knockdown of GATA4 did not reverse α1A survival signaling in α1B-single knockout cardiac myocytes, which express only the α1A-subtype and are protected from NE-induced cell death. In addition, we found that α1A stimulation did not increase phosphorylation of GATA4 or increase Bcl-2 levels, a survival factor regulated by GATA4 (11). We also observed that phosphorylation of GATA4 was not affected by aortic constriction and displayed a discordant regulation with phosphorylation of ERK. Furthermore, we found that α1-stimulation induced phospho-(activated)-ERK localization to the plasma membrane in adult cardiac myocytes, whereas phospho-ERK was detected throughout the cell following α1-stimulation in neonatal cardiac myocytes. This could possibly explain the lack of α1-mediated phosphorylation of the transcription/survival factor GATA4 in adult cardiac myocytes. In summary, our findings suggest that GATA4 is not downstream of α1A-ERK signaling in adult mouse cardiac myocytes and is not required for α1A survival signaling.

Our results, indicating that GATA4 is not required for α1A-survival signaling in adult mouse cardiac myocytes are surprising given that several studies identify GATA4 as a downstream effector of α1-mediated transcriptional activity, in both cultured neonatal rat cardiac myocytes and neonatal mice in vivo (2, 13, 15). Furthermore, previous studies indicate that GATA4 is required for α1-mediated protection in neonatal cardiac myocytes (2). While it is possible that the 62% reduction in GATA4 protein observed in our study (Fig. 2B) still allowed for normal GATA4 function in α1BKO cardiac myocytes, several lines of evidence support our assertion that GATA4 is not downstream of α1A-ERK signaling. First, previous reports suggest that GATA4 haploinsufficiency in mice increases susceptibility to doxorubicin-induced myocyte death (2), but we saw no effect of reducing GATA4 on α1A-survival signaling (Fig. 2). Second, we found no evidence that α1A-AR activation led to phosphorylation and activation of GATA4 in cultured adult mouse cardiac myocytes or adult mouse heart, although α1-AR activation induced phosphorylation of GATA4 in neonatal rat cardiac myocytes (Figs. 3 and 4). Third, we found that in adult mouse cardiac myocytes, α1-AR activation induced localization of phospho-ERK at the plasma membrane, not the nucleus, which could explain the failure of α1-ARs to activate GATA4 in adult cardiac myocytes (Fig. 5). Therefore, these results would suggest a critical difference in α1-AR signaling between adult and neonatal cardiac myocytes.

GATA4 protects cardiac myocytes from cell death by increasing the expression of survival factors such as Bcl-2 and Bcl-XL (2-4). In our experiments, we found that a short-term (2 h) exposure to NE increased cardiac myocyte death in α1ABKO myocytes. Further, we demonstrated that although the α1A-subtype prevented NE-induced cell death, GATA4 was not required for this effect. A 2-h exposure to NE would not be sufficient to upregulate gene expression; therefore, a failure to activate basal α1A-GATA4-mediated expression of survival factors like Bcl-2 or Bcl-XL (in the 40-h period prior to NE treatment) could explain our results. However, our failure to see any changes in GATA4 phosphorylation or change in Bcl-2 levels with long-term (24 h) α1-AR stimulation with phenylephrine would indicate that α1-AR signaling does not proceed through GATA4. Therefore, we are currently investigating other proposed mechanisms for α1A-survival signaling. Valks et al. (24) demonstrated that α1-AR stimulation induced the phosphorylation of the Bcl-2 family member Bad in neonatal rat cardiac myocytes (24). In many cell types, the phosphorylation of Bad is thought to reduce its proapoptotic activity by inducing binding to 14-3-3, thereby preventing Bad translocation to the mitochondrial membrane and subsequent interaction with Bcl-2 or Bcl-XL (25). Alternatively, Pu et al. (21) found that α1-mediated activation of NFAT transcription factors is critical in mediating survival signaling in neonatal rat cardiac myocytes. However, it is unclear whether either of these mechanisms are relevant in adult cardiac myocytes.

In summary, we identified GATA4 as a survival factor in adult mouse cardiac myocytes. However, our results indicate that GATA4 is not downstream of α1A-ERK signaling in adult mouse cardiac myocytes and that GATA4 is not required for α1A-survival signaling. By demonstrating that GATA4 is not required for α1A-survival signaling in adult cardiac myocytes, we also identified an important mechanistic difference in α1-AR signaling between adult and neonatal cardiac myocytes. Finally, the identification of the mechanisms by which α1-ARs protect the heart from stress is important in light of both ALLHAT and V-HeFT, which demonstrate that α1-blockade worsens heart failure and increases mortality (1, 7).

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