Disruption of Sarcolemmal ATP-Sensitive Potassium Channel Activity Impairs the Cardiac Response to Systolic Overload

Xinli Hu, Xin Xu, Yimin Huang, John Fassett, Thomas P. Flagg, Ying Zhang, Colin G. Nichols, Robert J. Bache, Yingjie Chen

Abstract—Sarcolemmal ATP-sensitive potassium channels (K_{ATP}) act as metabolic sensors that facilitate adaptation of the left ventricle to changes in energy requirements. This study examined the mechanism by which K_{ATP} dysfunction impairs the left ventricular response to stress using transgenic mouse strains with cardiac-specific disruption of K_{ATP} activity (SUR1-tg mice) or Kir6.2 gene deficiency (Kir6.2 KO). Both SUR1-tg and Kir6.2 KO mice had normal left ventricular mass and function under unstressed conditions. Following chronic transverse aortic constriction, both SUR1-tg and Kir6.2 KO mice developed more severe left ventricular hypertrophy and dysfunction as compared with their corresponding WT controls. Both SUR1-tg and Kir6.2 KO mice had significantly decreased expression of peroxisome proliferator-activated receptor γ coactivator (PGC)-1α and a group of energy metabolism related genes at both protein and mRNA levels. Furthermore, disruption of K_{ATP} repressed expression and promoter activity of PGC-1α in cultured rat neonatal cardiac myocytes in response to hypoxia, indicating that K_{ATP} activity is required to maintain PGC-1α expression under stress conditions. PGC-1α gene deficiency also exacerbated chronic transverse aortic constriction–induced ventricular hypertrophy and dysfunction, suggesting that depletion of PGC-1α can worsen systolic overload induced ventricular dysfunction. Both SUR1-tg and Kir6.2 KO mice had decreased FOXO1 after transverse aortic constriction, in agreement with the reports that a decrease of FOXO1 can repress PGC-1α expression. Furthermore, inhibition of K_{ATP} caused a decrease of FOXO1 associated with PGC-1α promoter. These data indicate that K_{ATP} channels facilitate the cardiac response to stress by regulating PGC-1α and its target genes, at least partially through the FOXO1 pathway. (Circ Res. 2008;103:1009-1017.)

Key Words: ATP-sensitive potassium channels ■ cardiac hypertrophy ■ PGC-1α

A TP-sensitive potassium channels (K_{ATP}) act as metabolic sensors that can regulate cellular activity to meet energetic demands.1 In the cardiac myocyte, K_{ATP} channels are composed of the pore forming subunit Kir6.2 and the regulatory subunit SUR2A. Patients with missense or frameshift mutations in genes encoding the cardiac K_{ATP} channel are predisposed to cardiomyopathy or sudden death.2,3 Moreover, in genetically modified mouse models, global Kir6.2 gene knockout (Kir6.2 KO) resulted in abolition of ischemic preconditioning.4 Reduced exercise capacity,5 impaired the response to adrenergic challenge,6 compromised the tolerance to hypertension,7 and impaired the ability to tolerate hemodynamic overload produced by transverse aortic constriction (TAC).8 Two recent studies using Kir6.2 KO mice reported that disruption of K_{ATP} channel activity led to activation of calcium-dependent calcineurin pathways, which, in turn, increased nuclear accumulation of the prohypertrophic transcription factors MEF2 and NFAT.7,8 However, the molecular mechanisms by which K_{ATP} channels regulate cardiac function, particularly during adaptation of the heart to the increased metabolic requirements produced by hemodynamic overload, are largely unknown. Most importantly, as a well defined metabolic stress sensor, the role of K_{ATP} channels in regulation of genes related to myocardial metabolism has not been studied.

At the transcriptional level, several families of transcription factors have been identified that regulate energy production processes, including peroxisome proliferator-activated receptor (PPAR),9 estrogen-related receptor (ERR),10,11 nuclear respiratory factor (NRF),12 Tfam (mitochondrial transcription factor A),13 PPARγ coactivator-1α (PGC-1α), and PGC-1β.14 PGC-1α and PGC-1β bind to both nuclear receptors and nonnuclear receptors and control cellular energy metabolic pathways. In transgenic mice, overexpression of

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Materials and Methods
SUR1-tg mice, Kir6.2 KO mice, and PGC-1α KO mice (gifts from Dr Daniel Kelly) were used in this study according to a protocol approved by the University of Minnesota Institutional Animal Care and Use Committee. Transverse aortic constriction (TAC) was used to create systolic pressure overload as previously described. An expanded Methods section is available in the online data supplement at http://circres.ahajournals.org.

Results
Disruption of K_{ATP} Channels Exacerbated Left Ventricular Hypertrophy and Dysfunction Produced by TAC
Similar to previous reports, mice with disrupted cardiac K_{ATP} channel activity secondary to cardiac-specific SUR1 overexpression develop and grow similarly to their wild-type (WT) littermates under normal conditions and had normal cardiac function under unstressed conditions (Table I in the online data supplement). Left ventricular (LV) dimensions and ejection fraction of the Kir6.2 KO were also similar to their WT control mice under unstressed conditions, consistent with previous reports. The sustained TAC will increase myocardial ATP demands and may compromise coronary perfusion to result in activation of K_{ATP} channels; consequently, we performed TAC to stress the hearts of SUR1-tg and WT. As expected, 4 weeks of severe TAC produced significant ventricular hypertrophy in both WT and SUR1-tg mice; however, the degree of hypertrophy assessed by heart weight-to-body weight ratio was ~15% greater in the SUR1-tg mice (Figure 1A). In addition, the cardiomyocyte cross-sectional area following TAC was significantly larger in SUR1-tg than in WT littermates (427 ± 26 μm² versus 359.3 ± 14.4 μm², respectively, P < 0.05). Both lung weight and lung weight-to-body weight ratio were significantly greater in the SUR1-tg mice as compared with WT following TAC (Figure 1B), indicating that SUR1-tg mice had more pulmonary congestion. Moreover, there was more LV dilation and greater impairment of LV function in SUR1-tg mice, with a greater decrease of LV ejection fraction than in the WT (Figure 1C through 1E, supplemental Table I).

Consistent with a greater degree of LV dysfunction, myocardial atrial natriuretic peptide (ANP) was significantly higher at both protein and mRNA levels in the SUR1-tg mice as compared with WT littermates after TAC (Figure 1F and 1G and supplemental Figure I). The expression level of Kir6.2 was not altered in response to TAC or ectopic expression of SUR1 (supplemental Figure I).

To confirm that the greater ventricular hypertrophy and dysfunction in SUR1-tg mice was indeed attributable to loss of K_{ATP} channel activity, we subsequently studied Kir6.2 KO mice. After 6 weeks of moderate TAC, Kir6.2 KO mice also developed more severe cardiac hypertrophy as compared with...
the WT mice (Figure 2A), and this was associated with a small but significant increase of the ratio of lung weight-to-body weight in Kir6.2 KO mice (Figure 2B). There was also a trend toward a greater decrease of LV ejection fraction in the Kir6.2 KO mice, but this difference was not significant (Figure 2C and 2D). Kir6.2 KO also exacerbated the increase of myocardial ANP produced by TAC (Figure 2E). The mortality after TAC was not different between Kir6.2 KO and WT mice.

Taken together, these data demonstrate that disruption of KATP channels exacerbated TAC-induced LV dysfunction and indicate that KATP channel activity is important for the compensatory responses that allow the heart to adapt to chronic systolic overload.

**Disruption of KATP Channels Attenuated Myocardial PGC-1α Expression in Response to Chronic Pressure Overload**

Studies of the Kir6.2 KO mice indicate that the presence, and presumably activation, of KATP channels during metabolic stress helps to preserve cellular ATP and maintain energy homeostasis. To determine whether disruption of KATP channels in the SUR1-tg mice altered components of energy producing systems, we examined the expression of enzymes related to myocardial ATP production. We found that uncoupling protein-3 (UCP3), cytochrome c, cytochrome c oxidase subunit-III (COX-III), and carnitine palmitoyltransferase-1 muscle isoform (CPT-1b) were each significantly decreased in the SUR1-tg mice both under control conditions and after TAC (Figure 3A through 3C and supplemental Figure II), indicating that abnormal mitochondrial function might contribute to the LV dysfunction that we observed in the SUR1-tg mice after TAC. Furthermore, the mRNA content of very long chain acetyl-CoA dehydrogenase (VLCAD), medium chain acetyl-CoA dehydrogenase (MCAD), CPT-1b, COX-I, and COX-III were all significantly decreased in the SUR1-tg mice as compared with WT littermates under both control conditions and after TAC (Figure 3D and supplemental Figure III). Similarly, TAC caused significantly decreased...
expression of these enzymes in the Kir6.2 KO mice as compared with the WT mice (Figure 3E). These results indicate that disrupting KATP channel activity attenuated the expression of mitochondrial energy metabolism related enzymes. However, there was no significant difference in myocardial mitochondrial volume density between the WT and SUR1-tg mice before or after TAC, suggesting that the quality, but not the quantity, of mitochondria was changed in the SUR1-tg hearts (supplemental Figure IV).

We also examined upstream transcriptional factors that have been shown to regulate energy metabolism related genes, including PGC-1α, PGC-1β, ERRα, ERRγ, PPARα, PPARγ, Tfam, and nuclear respiratory factor-1. TAC caused significant up regulation of PGC-1α and PPARα in WT, although this induction was abolished by ablation of KATP activity in the SUR1-tg mice (Figure 4A). Interestingly, myocardial PGC-1α protein content was significantly decreased in SUR1-tg mice compared with WT mice under both control conditions and after TAC (Figure 4B and 4C). In addition, TAC caused significantly greater decreases of myocardial PGC-1α mRNA in Kir6.2 KO mice as compared with their WT littermates (Figure 4D). These data indicate that KATP channel dysfunction leads to deregulation of PGC-1α expression and its downstream target genes in response to systolic overload.

Blocking KATP Channel Activity in Rat Neonatal Cardiomyocytes Decreased the Promoter Activity and mRNA Level of PGC-1α

A decrease in myocardial PGC-1α content has been reported in several heart failure models.17,26 However, changes of PGC-1α expression in vivo could be the result of altered neurohormonal signaling in the setting of heart failure, rather than a direct effect of KATP activity. To determine whether inhibition of KATP channel activity can directly affect PGC-1α expression in cardiac myocytes, we studied the effect of inhibiting KATP channels on PGC-1α expression in rat neonatal cardiac myocytes. Because cardiac KATP channels are likely to be closed in cultured cardiac myocytes under basal conditions, we challenged the cells with hypoxia/reoxygenation (H/R) to activate KATP channels (24 hours of 1% oxygen followed by 7 to 8 hours of reoxygenation). KATP channel activity was suppressed either pharmacologically with glibenclamide or by selective gene silencing of cardiac KATP regulatory subunit, SUR2A. Both pharmacological and genetic suppression significantly repressed the expression of PGC-1α at the mRNA level (Figure 5A and 5B). Expression of the 2 downstream targets of PGC-1α genes, CPT-1b and VLCAD, was also determined. The mRNA level of CPT-1b was significantly reduced by glibenclamide treatment or SUR2 gene silencing. The expression of VLCAD also tended to decrease after glibenclamide treatment (Figure 5A and 5B).
In a subsequent study, rat neonatal cardiomyocytes were transfected with a luciferase reporter driven by a 3.1-kb mouse PGC-1α promoter. Either glibenclamide treatment or knocking down SUR2 expression significantly reduced reporter activity by ∼22% and 32%, respectively (Figure 5C and 5D), suggesting that blocking K_ATP channel activity can repress expression of PGC-1α at the transcriptional level.

**PGC-1α KO Exacerbated Left Ventricular Hypertrophy and Dysfunction Produced by Moderate TAC**

To determine whether a decrease of PGC-1α can contribute to pressure overload induced myocardial hypertrophy and dysfunction, we determined ventricular structure and function to pressure overload induced myocardial hypertrophy and dysfunction, we determined ventricular structure and function to pressure overload induced myocardial hypertrophy and dysfunction.

**Disruption of K_ATP Activity in the SUR1-tg Mice**

To confirm this, we prepared nuclear extract from flash frozen heart samples. The SUR1-tg mice had a significantly decreased nuclear FOXO1 as compared with the sham group and WT banded mice. Interestingly, nuclear FOXO1 was slightly increased in the WT mouse heart following TAC but decreased in the SUR1-tg heart under basal conditions, although neither was statistically significant. The amount of cytoplasmic fraction of FOXO1 was not different among the 4 groups (Figure 7C). Similarly, TAC resulted in significantly decreased expression of both PGC-1α and FOXO1 in the Kir6.2 KO mice but not in WT mice (Figure 7D). Taken together, these data suggest that decreased FOXO1 might partially account for the repressed expression of PGC-1α when K_ATP channel activity is disrupted.

We further examined the levels of Akt and FOXO1 and the subcellular distribution of FOXO1 in neonatal cardiomyocytes subjected to H/R, with or without disruption of K_ATP. Under normoxic conditions, glibenclamide or silencing SUR2 gene expression did not activate Akt or change FOXO1 expression. Under hypoxic conditions, the amount of phospho-AktSer473 was increased when K_ATP channels were pharmacologically blocked with glibenclamide or silencing SUR2 gene expression (Figure 7E). Furthermore, K_ATP channel blockade significantly decreased the amount of FOXO1 in nuclei, whereas cytosolic FOXO1 was significantly increased. These data indicate that blocking K_ATP channel activity under stress conditions leads to decreased nuclear FOXO1 and that increased phospho-AktSer473 may be one of the factors that induces nuclear exclusion of FOXO1 when K_ATP is disrupted.

**Mutations of Potential FOXO1 Binding Sites on PGC-1α Promoter Abolished H/R-Induced PGC-1α Promoter Activity**

To determine whether reduced nuclear FOXO1 leads to repression of PGC-1α promoter activity, point mutations were introduced into each IRS in the luciferase reporter construct and reporter activities were determined (Figure 8A and 8B). After H/R, the promoter activity of PGC-1α was significantly decreased when the IRSs were mutated, indicating that these IRSs are important in maintaining PGC-1α promoter activity. Glibenclamide treatment further decreased the luciferase activity of the mutated reporters, suggesting that K_ATP channels might influence the PGC-1α promoter through other
pathway(s) in addition to FOXO1. Finally, chromatin immuno-
precipitation assay was performed using anti-FOXO1 antibody
(Figure 8C). Treatment with glibenclamide or knockdown of
SUR2 subunit expression decreased the amount of FOXO1
associated with the IRSs. Taken together, these data indicate that
inactivation of KATP activity under stress conditions represses
PGC-1α expression, at least partially through dissociation of the
positive regulator FOXO1 from its promoter.

Discussion
The major finding of this study is that K_ATP channels can
regulate energy metabolism related gene expression through
PGC-1α. To our knowledge, this provides the first evidence that
functional K_ATP channels influence the expression of
myocardial PGC-1α and the expression of a group of proteins
related to ATP production. Thus, K_ATP channels play a critical
role in augmenting the myocardial energy supply during
chronic hemodynamic overload by regulating PGC-1α ex-
pression at the transcriptional level.

In this study, we used cardiac-specific SUR1-tg mice in
which ventricular K_ATP channel activity is essentially abol-
ished as the result of ectopic expression of the SUR1
regulatory subunit.22 Using cardiac-specific disruption of
K_ATP channels avoids potential unwanted systemic effects of
global gene deletion such as insulin secretion defects\textsuperscript{28} or coronary spasm\textsuperscript{29} that have been described in other global K\textsubscript{ATP} gene-deficient mouse strains. To confirm that our observations were caused by K\textsubscript{ATP} ablation, we also studied the effects of global depletion of the pore forming subunit in Kir6.2 KO mice. Our findings that disruption of K\textsubscript{ATP} exacerbated the TAC induced cardiac hypertrophy and dysfunction in both the SUR1-tg and the Kir6.2 KO mice are consistent with a previous report that disruption of K\textsubscript{ATP} impaired the tolerance of Kir6.2 KO mice to TAC-induced systolic overload. Those investigators reported that in response to TAC, action potential duration shortened in WT mice but was prolonged in Kir6.2 KO mice, a response that resulted in calcium overload in the Kir6.2 KO myocytes.\textsuperscript{8} Interestingly, we now show that suppression of K\textsubscript{ATP} channel activity during TAC also disrupts the expression of PGC-1\textalpha/\textsuperscript{25} via the FOXO1 signaling pathway. The link between K\textsubscript{ATP} channels and regulation of this master metabolic regulatory pathway remains to be determined, but several studies have demonstrated that $[\text{Ca}^{2+}]_{\text{i}}$ is able to regulate Akt activity.\textsuperscript{30,31} It is conceivable that disruption of K\textsubscript{ATP} channel activity led to Ca\textsuperscript{2+} overload in the cardiomyocytes,\textsuperscript{32} which, in turn, contributed to activation of Akt. The results of the present study demonstrate that K\textsubscript{ATP} channel activity affects the expression of metabolism related enzymes important for ATP production. In a recent study, Jilkina et al observed that the baseline ATP level in Kir6.2 KO hearts was decreased by 30\%, and this difference was even more evident when the heart was stressed by metabolic inhibition or isoproterenol infusion.\textsuperscript{33} We demonstrate here that some myocardial metabolic enzymes were decreased in unstressed SUR1-tg and Kir6.2 KO mice, suggesting that K\textsubscript{ATP} activity controls the expression of metabolic enzymes during control conditions. The depression of PGC-1\textalpha signaling as a result of K\textsubscript{ATP} suppression is likely to reduce the myocardial metabolic reserve available for energy production during increases of cardiac work\textsuperscript{34} and may, thereby, provide a mechanistic basis for the link between K\textsubscript{ATP} activity and ATP production reported in Kir6.2 KO mice.

Previous studies have shown that defects of mitochondrial respiratory chain complexes can lead to LV dysfunction or cardiomyopathy.\textsuperscript{35} Similarly, 2 PGC-1\textalpha knockout mouse models exhibited differing severities of cardiac phenotype, but both showed impairment of the cardiac response to stress (TAC, dobutamine challenge, or exhaustive exercise),\textsuperscript{16,17,23} suggesting that PGC-1\textalpha becomes more critical during stress conditions, consistent with our findings of greater LV dysfunction and pulmonary congestion in PGC-1\textalpha-null mice.
following TAC. It should be noted that in the SUR1-tg study, PGC-1α mRNA was increased, whereas the PGC-1α protein was decreased in the WT mice after TAC. This disparity between mRNA and protein expression suggests that the expression of PGC-1α is regulated at the translational and/or posttranslational level in response to pressure overload. Our finding that PGC-1α mRNA was increased in WT mice after TAC is contrary to reports that TAC caused a decrease of myocardial PGC-1α.17,26 This discrepancy may be the result of differences in the degree of LV dysfunction or the duration of TAC. However, TAC caused more ventricular hypertrophy and a greater decrease of PGC-1α in both SUR1-tg and Kir6.2 KO mice as compared with their corresponding WT littermates, indicating that intact KATP activity is important for maintaining myocardial PGC-1α expression and ventricular function when the heart is stressed.

Our previous studies consistently show that TAC induces myocardial Akt activation.24,25 Several studies using overexpression of activated Akt demonstrate that short-term Akt activation results in “physiological” hypertrophy, but chronic, unregulated Akt activation in the heart can be detrimental.36,37 Thus, both the extent and duration of Akt signaling are important in regulating cardiac function. Cardiac-specific overexpression of a constitutively active mutant of Akt (myr-Akt) leads to ≈2-fold reduction in expression of PPARα and PGC-1α mRNA, suggesting that Akt may regulate the expression of these 2 genes, presumably through the phosphorylation and nuclear exclusion of phos-FOXO1.38 In the present study, we observed increased phos-Akt in the SUR1-tg mice relative to WT in both unstressed and stressed conditions. In addition, disruption of KATP channel activity in neonatal cardiac myocytes enhanced phosphorylation of Akt (Figure 7E). However, the activation of Akt did not correlate very well with FOXO1 protein levels in the SUR1-tg study. One possible explanation for this is that other signaling pathways that induce the nuclear retention of FOXO139 might overcome the Akt signaling that induces cytoplasmic localization of FOXO1. To this end, our results support the concept that disruption of KATP channels enhanced TAC-induced Akt activation. However, whether the increased Akt activation contributed to the decreased PGC-1α is uncertain. The combined insult of TAC and KATP suppression in the SUR1-tg and Kir6.2 KO mice led to a decrease in total FOXO1, suggesting a synergistic effect of metabolic stress and KATP channel activity on FOXO1 protein level.

We used rat neonatal cardiomyocytes to further characterize the effect of KATP channels on PGC-1α. Because the aim was to determine whether inhibition of KATP channel activity would depress PGC-1α expression, we exposed the cells with H/R to activate the KATP channels. Suppression of KATP channel activity either with glibenclamide or by selective gene silencing of SUR2 significantly repressed expression of PGC-1α and its downstream target gene CPT-1b at mRNA level. Furthermore, both glibenclamide and knocking down SUR2 expression significantly reduced reporter activity driven by PGC-1α promoter, indicating that KATP channels are able to influence PGC-1α expression at the transcriptional level. The studies in isolated cardiomyocytes were designed to examine whether the regulation of PGC-1α expression by KATP channels might be a general response of heart under stress conditions. Although challenging cultured cells with H/R can effectively manipulate cardiomyocyte KATP activity in the absence of changes in neurohormonal agents that occur in vivo, it does not cause myocyte hypertrophy. Therefore, the H/R study is not a specific or ideal model to mimic the in vivo pressure overload induced ventricular hypertrophy, which is a limitation of this study. However, it should be noted that Sano et al demonstrated that TAC does cause myocardial hypoxia in vivo, likely attributable to decreased vascular density in conjunction with increased metabolic demands secondary to increased cardiac work.30

Taken together, using 2 mouse strains in which ventricular KATP channel activity was disrupted, we demonstrate that loss of KATP channel activity attenuated the expression of PGC-1α and mitochondrial energy metabolism–related enzymes in the heart and exacerbated TAC-induced ventricular hypertrophy and dysfunction, indicating a role for KATP channels in the chronic response to increased cardiac work. We provide further evidence that the protective effect of KATP channels occurs at least partially by regulating the activity of FOXO1, which, in turn, influences the expression of PGC-1α and its downstream target genes. Our findings support an important role for KATP channels in regulating the myocardial expression of metabolism-related genes in the cardiac response to hemodynamic overload (Figure 8D).

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Disclosures
None.

References
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**Material and Methods**

**Animals and Transverse Aortic Constriction (TAC)**

Adult (12-15 weeks) high-expressing Line 720 SUR1-tg mice and their WT littermates were used in this study. Kir6.2 KO mice backcrossed for five generations into a C57BL/6 background were further crossed into a C57BL/6 background two additional times to generate heterozygous Kir6.2 mice as breeders. The homozygous Kir6.2 KO and wild type littermates produced were used for the study. PGC1α KO mice were generated as previously described. All animal studies were performed according to a protocol approved by the University of Minnesota Institutional Animal Care and Use Committee. Severe TAC (using a 27G needle to calibrate the degree of aortic constriction) was performed in SUR1-tg mice as described. Previous studies demonstrated that severe TAC caused a high mortality rate in both Kir6.2 KO and PGC1α KO mice; consequently, to allow a 6 week observation period in these strains, moderate TAC (using a 26G needle to calibrate the degree of aortic constriction) was performed. Echocardiography was performed while the mice were anesthetized with 1.5% isoflurane by inhalation.

**Western Blots and Quantitative Real-time PCR**

Primary antibody against ANP was from Peninsula Lab., Bachem AG; antibodies against PGC-1α, COXI, COXIII, UCP3, and SUR2A were from Santa Cruz Biotech; antibodies against cytochrome C, phos-Akt<sup>Ser472</sup>, total-Akt, phos-FOXO1<sup>Thr24</sup>, total-FOXO1 were from Cell Signaling, and antibody against CPT-1b was from Alpha
Diagnostics. HRP conjugated secondary antibodies were from BioRad Laboratories and Sigma.

Total RNA was reverse-transcribed using random hexamers and Moloney murine leukemia virus (MMLV) reverse transcriptase (Clontech). The real-time PCR reaction was carried out using the Light Cycler Thermocycler (Roche Diagnostics Corp). Primers are listed in Online Table II. Results were normalized to $\alpha$-sarcomeric actin.

**Cell Culture, Transfection, Reporter Gene Assay**

Rat neonatal cardiomyocytes were isolated and cultured as described previously. Reporter genes were transfected with Lipofectamine 2000 (Invitrogen) and SUR2 specific siRNA or ON-TARGETplus siCONTROL. Non-Targeting siRNA (Dharmacon) were transfected with DharmaFECT 3 reagent (Dharmacon) into neonatal cardiomyocytes. Forty-eight hours after transfection, cells were treated with 50$\mu$M glibenclamide or vehicle (DMSO) for 45 minutes and then subjected to 24 hours of hypoxia (1% $O_2$) followed by 7-8 hours of reoxygenation. After hypoxia/reoxygenation (H/R), cells were harvested for reporter assay or for real-time PCR and Western blot analysis.

Reporter constructs were generated by inserting 3.1Kb PGC-1$\alpha$ promoter into pGL3B vector (Promega). The mutations were introduced into the wild type reporter using QuickChange Multi Site-Directed Mutagenesis Kit (Stratagene). LacZ reporter driven by CMV promoter was used as an internal control for transfection, and relative luciferase activity was determined by normalizing luciferase activity to the corresponding LacZ activity.
Preparation of Nuclear Extract

Frozen heart tissue was ground in liquid nitrogen into fine power and homogenized in hypotonic buffer. Rat neonatal cardiomyocytes were washed in DPBS (Invitrogen) and resuspended in hypotonic buffer. Then nuclear extract was prepared as described\textsuperscript{9}.

ChIP Assay

ChIP assay was performed as described\textsuperscript{10}. DNA was purified from the immunocomplex precipitated with anti-FOXO1 antibody and subjected to real-time PCR. Chromatin solutions with non-specific IgG were used as control for total input.

Statistical Analysis

Results are expressed as mean ± standard error of the mean (SEM). The number of samples in each group for each experiment was at least six or as indicated in the legends. An unpaired two-tailed Student’s t-test was used to determine $p$ values. A $p$ value of $\leq 0.05$ was considered significant.
References


Online Table I. Disruption of K<sub>ATP</sub> channel activity exacerbated LV dysfunction in response to TAC

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<th>WT sham (n=10)</th>
<th>WT TAC (n=10)</th>
<th>SUR1-tg sham (n=10)</th>
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* p<0.05 versus sham, # p<0.05 versus WT littermate
Online Table II. Primers used in real-time PCR, ChIP assay and point mutation

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<td>VLCAD (rat)</td>
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<td>5'-TGAGTGCAGTGTTGAGAG-3'</td>
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<tr>
<td>MCAD</td>
<td>5'-CTAACCCAGATGAAATCCCG-3'</td>
<td>5'-GGGTTCGGCTTTCAATGA-3'</td>
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<tr>
<td>CPT1b</td>
<td>5'-GAGTTTCGATGCTGGCTCCG-3'</td>
<td>5'-GACAGGACACTGTGGTTGAG-3'</td>
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<td>COX I</td>
<td>5'-TGAAACCCAGCCACAAATC-3'</td>
<td>5'-CCAGCGGGCATCAAAGAAG-3'</td>
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<td>COXIII</td>
<td>5'-CATGCTTTGAGATTTCTT-3'</td>
<td>5'-ATTAGTCCGCTTTATGGAG-3'</td>
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<td>PGC-1α</td>
<td>5'-AACTCAGAACTCTCCAGGGCT-3'</td>
<td>5'-TTAGTTCACTGTTCTTCT-3'</td>
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<tr>
<td>PGC-1α(rat)</td>
<td>5'-GGTTCAGGAGATCCTCCGCG-3'</td>
<td>5'-TTTGCAGTCCGCTTTATCA-3'</td>
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<td>PGC-1β</td>
<td>5'-CAACGTTTTCATACCTTGCC-3'</td>
<td>5'-GCTCATGTCGCCTTTTCT-3'</td>
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<td>ERRα</td>
<td>5'-TGGAGCGGGAGAGGATCGTC-3'</td>
<td>5'-CAGCTTCAGCATTTCAATGA-3'</td>
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<td>ERRγ</td>
<td>5'-CTCTGTGACTGGGCTGACCC-3'</td>
<td>5'-CCAGGGACAGTGGAGAAGC-3'</td>
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<tr>
<td>PPARα</td>
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<td>5'-CAGATGTTCAATGCAACACT-3'</td>
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<tr>
<td>PPARγ</td>
<td>5'-CAAGAGTAACTGCATCTCC-3'</td>
<td>5'-CCTTCAAGCATGAACTCC-3'</td>
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<td>Tfam</td>
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<td>5'-CCATGTTAAGGAAAACACT-3'</td>
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<td>NRF1</td>
<td>5'-GGCCGGGAGATTTTATATGCTTT-3'</td>
<td>5'-GGGCTTGATGCTTGCT-3'</td>
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<td><strong>ChIP Assay</strong></td>
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<td>5'-TCCCCATCGTACGGTTACGAC-3'</td>
<td>5'-GTGCCCTGGGTGACAGTTT-3'</td>
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<tr>
<td>Site 2</td>
<td>5'-ACTGCAAGGAGACAGCTGATTTG-3'</td>
<td>5'-CCCTATCTTCTTCTTGCTTT-3'</td>
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<tr>
<td>Site 3</td>
<td>5'-GGCAAGGATGCTAGCTACTTGTCA-3'</td>
<td>5'-CCAGGCTTGAATGCCCCACTCT-3'</td>
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<td><strong>Point Mutations</strong></td>
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<tr>
<td>5'-GATACCATTTCAGTGATGGATCCTCCTTACATCCCTGGA-3'(sense)</td>
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<tr>
<td>5'-AAACAAATGTTGGCCTGTAACCGTGAATAAACATAGGAA-3'(sense)</td>
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<tr>
<td>5'-TTGCTATTGGCCCTGTGGAGGAAATAATT-3'(sense)</td>
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</table>

Primers were designed according to mouse cDNA or genomic DNA sequences if not specifically labeled. For point mutations, only sense primers were used for mutagenesis of the reporter gene. The mutated nucleotides were in italic and underlined.
Online Figure I. Myocardial ANP expression was significantly increased in the SUR1-tg mice. The protein level of the pore forming subunit Kir6.2 was not changed in the SUR1-tg mice or in response to TAC.
Online Figure II. Ponseau S staining of the blots on PVDF membrane before Western blot for sample loading control of Figure 3A and Figure 3B. A, 25μg of total lysate resolved on 15% SDS-PAGE (gel used for Figure 3A); B, 75μg of total lysate resolved on 10% SDS-PAGE (gel used for Figure 3B).
Online Figure III. The mRNA levels of GAPDH in the SUR1-tg and WT mice after normalized to α-actin and 18SrRNA. The RT and real-time PCR results in this study were normalized to α-actin.
Online Figure IV. Following TAC the mitochondria volume density is not significantly different between the SUR1-tg and WT mice.