Roles of histone deacetylation and AMP kinase in regulation of cardiomyocyte PGC-1α gene expression in hypoxia

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The human heart utilizes ~6 kg of ATP per day, more than any other organ in the body (24). To achieve this high level of throughput, precise control over fuel production and consumption is required. While the general biochemical mechanisms responsible for normal energy metabolism have been defined in detail, it is only over the past decade that the transcriptional regulators of the responsible genes have begun to be elucidated. Arguably, one of the most important metabolic regulators identified to date is the peroxisome proliferator-activated receptor-γ (PPAR-γ) coactivator 1α (PGC-1α).

PGC-1α exerts numerous effects on cell metabolism depending on tissue type, including regulation of gluconeogenesis in the liver and fiber type determination in skeletal muscle (33, 44). In the heart, PGC-1α regulates the expression of genes that in turn regulate mitochondrial biogenesis and fatty acid β-oxidation (22, 35, 40). Overexpression or gene deletion of PGC-1α has significant effects on myocardial metabolism, resulting in increased respiration and mitochondrial synthesis or impaired responses to pressure overload, respectively (6, 30, 32). Conversely, loss of PGC-1α expression contributes to basal cardiac dysfunction and increased susceptibility to pressure overload-induced cardiac failure (6, 30, 32). Elucidating the factors that control PGC-1α expression is thus of paramount importance in understanding cardiac function in both health and disease.

PGC-1α expression is altered in various physiological and pathological states. Exercise induces PGC-1α expression in skeletal muscle, while cold induces PGC-1α expression in brown fat where it plays a central role in thermogenesis (2, 36). Increased PGC-1α expression in fasted liver regulates gluconeogenesis (44). Thus, in physiologic states marked by fuel shortage or increased energy demand, PGC-1α expression typically increases. In contrast, PGC-1α expression is reduced in pathologic states including cardiac diseases such as ischemia and heart failure, despite reductions in fuel availability that may otherwise be expected to induce PGC-1α expression (16, 31). It is thus likely that other factors are important in such states. One possible regulator of PGC-1α expression is oxygen availability.

In the present study we examined the effect of hypoxia on PGC-1α expression in primary rat cardiomyocytes. Our results indicate that PGC-1α expression undergoes biphasic regulation during early and late hypoxia of cardiomyocytes, resulting in decreased or increased expression, respectively, due to promoter histone deacetylation and AMP kinase (AMPK) activation. We also found that the metabolic gene transcriptional regulator estrogen-related receptor-α (ERRα) is sufficient to overcome hypoxia-mediated downregulation of PGC-1α mRNA. These results indicate that PGC-1α expression responds not only to fuel signals but to oxygen availability as well, which may help to explain how PGC-1α becomes downregulated in cardiac disease.

MATERIALS AND METHODS

Ethics statement. Animals were treated in accordance with the guidelines of the Canadian Council on Animal Care and approved by the University of Manitoba Animal Protocol Management and Review Committee. Rat hypoxia experiments were approved by the Institutional Animal Care and Use Committee at the University of Colorado Denver and conducted in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

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Hypoxia studies. For cell hypoxia studies, primary cardiomyocytes were isolated from 1-day-old neonatal rat pups via enzymatic digestion as previously reported; maintained in DMEM supplemented with 16.6% M199, 1% FBS, 10% horse serum, 1% penicillin/streptomycin, and 1% L-glutamine; and placed in an air-tight incubator with 95% N2-5% CO2 (1). Treatment of cells with pharmacological inhibitors [trichostatin A (TSA), XCT790, or compound C] was initiated 24 h before endpoint analysis or the initiation of hypoxia as required. For some experiments, cardiomyocytes were infected 24 h before hypoxia with adenovirus encoding either green fluorescent protein (Ad-GFP) or ERKs (Ad-ERKs) using a multiplicity of infection of 100. Adult male Sprague-Dawley rats were placed in a hypobaric chamber to simulate an altitude of 18,000 ft above sea level with 10% O2 for 3 wk or were maintained as normobaric normoxic controls (21% O2; Ref. 10). Adult cardiomyocytes were isolated from a male Sprague-Dawley rat (328 g) by Langendorff perfusion according to the method of Thandapilly et al. (43). Adult cardiomyocytes were allowed to stabilize for 2 h after isolation in DMEM/M199 as above and then made hypoxic as performed for neonatal cardiomyocytes.

Luciferase reporter assay. Luciferase reporter assays were performed as previously described (11). COST cells grown in six-well dishes were transfected as required at 70–80% confluence, using 1 μg total DNA/well. The PGC-1α promoter reporter vector was described previously (12). All samples were cotransfected with 5 ng renilla luciferase expression plasmid (pRL) for normalization. The luciferase activity of each sample was measured on a TD20/20 luminometer (Turner BioSystems).

Quantitative real-time PCR. RNA was isolated from neonatal (GenElute Mammalian Total RNA Miniprep Kit; Sigma) or adult (GeneJet RNA Purification Kit; Fermentas) rat cardiomyocytes as per manufacturer’s instructions. Twenty five nanograms (neonatal cells) or 100 ng (adult cells) RNA were used for real-time PCR reactions using a Quanta Biosciences B-R 1 Step SYBR qRT-PCR kit as per manufacturer’s instructions. The various primers used in this study are listed in Table 1 (29, 42). A Bio-Rad iQ5 real-time PCR thermocycler was used for the amplification reactions. Melt curve data were collected from 81 cycles of an initial annealing at 55°C followed by each succeeding cycle. The cycling and melt curve parameters were consistent with recommendations of the B-R 1 Step SYBR qRT-PCR kit. Data were normalized to GAPDH, and relative gene expression was calculated using the 2^ΔΔCT method.

Cell viability. Cell viability following hypoxia was assessed using trypan blue exclusion. Neonatal rat cardiomyocytes were washed twice with 1× PBS and harvested by gentle scraping. Cells were resuspended in PBS and then mixed 1:1 with 0.4% trypan blue (Sigma-Aldrich) and incubated for 3 min at room temperature. Unstained viable and stained nonviable cells were counted using a hemocytometer, and viability is expressed as a percentage of the total.

Reactive oxygen species quantification. Hypoxia-induced oxidative stress was assayed by quantification of reactive oxygen species using conversion of nonfluorescent 5-(6)-chloromethyl-2’7’-dichlorodihydrofluorescein diacetate (CM-H2DCFDA; Invitrogen) to fluorescent 2’7’-dichlorodihydrofluorescein (DCF). Normoxic or hypoxic cardiomyocytes were treated with a 10 μM solution of CM-H2DCFDA in PBS for 10 min at 37°C in the dark. Cells were washed once with PBS, and fluorescence intensity was measured using a Zeiss AxioImager M1 epifluorescence microscope, with an excitation wavelength of 485 nm and emission wavelength of 530 nm. Five randomly chosen fields were imaged from each of 3 separate samples for a total of 15 fields per data point. The fluorescence intensity of 20 randomly selected cells from each field was quantified using Axiovision software, resulting in 100 cells analyzed per data point per experiment.

Chromatin immunoprecipitation assay. Chromatin immunoprecipitation (ChiP) assay was performed as previously described, using antibodies specific to ERKs (Santa Cruz Biotechnology) or histone H3 acetylated on lysine 9 (H3K9; Upstate; Ref. 11). Nonspecific IgG antibody (Santa Cruz Biotechnology) was used for negative controls. Cell lysates were sonicated on ice (3 repetitions of 10 s pulses) to shear DNA to 500- to 200-bp fragments. A 1% aliquot of cell lysate was used as an input control. For quantification of ChiP results, amplicon band intensity was measured using Quantity One software (Bio-Rad).

Statistical analysis. Results represent means ± SE for at least three independent experiments. Statistical significance was calculated by Student’s t-test or one-way or repeated-measures ANOVA using Student-Newman-Keuls post hoc analysis, with P < 0.05 considered significant.

RESULTS

PGC-1α is downregulated in hypoxic cardiomyocytes. Hypoxia decreases fatty acid oxidation in cardiomyocytes in part by downregulating PPARs-mediated gene expression (23). Since PGC-1α is a critical coregulator of PPARs target genes including those governing fatty acid oxidation, we hypothesized that loss of PGC-1α expression may also occur during hypoxia when oxygen, but not fuel, is limiting. Neonatal rat cardiomyocytes were made hypoxic (95% N2-5% CO2) in glucose-containing culture medium, resulting in increased oxidative stress as measured by a time-dependent increase in DCF fluorescence peaking within 6 h (Fig. 1A). Hypoxia did not induce cell death as measured by trypan blue exclusion (Fig. 1B), thus our subsequent results are not due to cell loss. Compounds used in later studies (TSA, XCT790) did not affect viability.

We examined the effect of hypoxia on PGC-1α mRNA expression, which decreased significantly starting at 9 h of hypoxia and reaching nearly 50% inhibition by 12 h (Fig. 1C). Similar significant decreases were observed in the expression of both major PGC-1α isoforms at 12 h of hypoxia (PGC-1α-A and PGC-1α-B; data not shown). Although the present study focused on transcriptional regulation, we also examined PGC-1α protein. At the relatively short time points employed in this study, we did not observe decreased PGC-1α protein expression (data not shown).

PGC-1α downregulation during hypoxia is due to histone deacetylation. There is mounting evidence that histone deacetylase (HDAC) activity is augmented during times of oxygen scarcity. HDACs positively regulate angiogenesis in response to hypoxia, and derepression of MDR1 expression during

Table 1. Sequences of forward and reverse primers employed

<table>
<thead>
<tr>
<th>Primer</th>
<th>Sequence (5′–3′)</th>
<th>Usage</th>
</tr>
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<tr>
<td>PGC-1α (fwd)*</td>
<td>AATGGTCGAATCTTCGTGGAACTGCT</td>
<td>qPCR</td>
</tr>
<tr>
<td>PGC-1α (rev)*</td>
<td>GGTTATGCTTGGGCTGTTCGGTTATG</td>
<td>qPCR</td>
</tr>
<tr>
<td>ERKs (fwd)*</td>
<td>ACTGCCACTGCAAGTAGAG</td>
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</tr>
<tr>
<td>ERKs (rev)*</td>
<td>CACAGGCTCAGACATCCA</td>
<td>qPCR</td>
</tr>
<tr>
<td>PGC-1α-A (fwd)†</td>
<td>GGGAATGTCGCTGCCAGA</td>
<td>qPCR</td>
</tr>
<tr>
<td>PGC-1α-A (rev)†</td>
<td>AGGAGTGTCGCTCAGCCA</td>
<td>qPCR</td>
</tr>
<tr>
<td>PGC-1α-B (fwd)†</td>
<td>GATATGGATGTCGGGTTTGTCA</td>
<td>qPCR</td>
</tr>
<tr>
<td>PGC-1α-B (rev)†</td>
<td>ACTGAAGTGCCTGGGGGTTTCCA</td>
<td>qPCR</td>
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<tr>
<td>GAPDH (fwd)</td>
<td>TCAGACACCAATCCTTCGGAGC</td>
<td>qPCR</td>
</tr>
<tr>
<td>GAPDH (rev)</td>
<td>GGCATGATGTCGTGGTATAG</td>
<td>qPCR</td>
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PGC-1α, peroxisome proliferator-activated receptor-γ coactivator-1α; ERKs, estrogen-related receptor-α; qPCR, quantitative PCR; fwd, forward; rev, reverse. *Sequences derived from Lagueau et al. (29). †Sequences derived from Tadaishi et al. (42).
hypoxia is HDAC5 dependent (25, 34). Recently it was reported that cardiac HDAC5 expression significantly increases during anoxia (27). We previously demonstrated that overexpression of constitutively nuclear histone deacetylase 5 (HDAC5S/A) inhibited PGC-1α expression (12), thus raising the possibility that loss of PGC-1α expression is due to increased HDAC activity during hypoxia. We therefore examined the acetylation status of histone H3K9 within the PGC-1α promoter during hypoxia using ChIP. Hypoxia significantly decreased histone H3K9 acetylation starting at 6 h of hypoxia, i.e., 3 h before the observed reduction in PGC-1α mRNA expression in response to hypoxia (Figs. 1C and 2, A and B). Blocking HDAC activity with the pan-HDAC inhibitor TSA during hypoxia completely attenuated loss of PGC-1α (Fig. 2C), implicating HDAC activation as the main regulator of PGC-1α downregulation. TSA treatment prevented PGC-1α promoter H3K9 deacetylation at 12 h of hypoxia (Fig. 2D).

PGC-1α expression is restored upon reoxygenation. To examine PGC-1α expression during reoxygenation, we transiently transfected the PGC-1α promoter reporter into neonatal cardiomyocytes. Hypoxia significantly inhibited PGC-1α promoter activity, in agreement with our earlier experiments (Fig. 3A). Reoxygenation significantly increased promoter activity but not to prehypoxia levels. Nonetheless, actual PGC-1α mRNA expression was normalized after 12-h reoxygenation, with a further significant increase at 24 h (Fig. 3B). We hypothesized that ERRα may play a role in the recovery of PGC-1α mRNA expression based on data from our laboratory and others indicating that ERRα regulates PGC-1α expression (5, 9, 37). However, blockade of ERRα activity during reoxygenation with the ERRα-specific inverse agonist XCT790 did not affect recovery (Fig. 3C). This result suggests that an ERRα-independent mechanism is responsible for PGC-1α restoration during reoxygenation.

Hypoxia-mediated downregulation of PGC-1α is attenuated by ERRα overexpression. Our laboratory has previously demonstrated that ERRα overexpression is sufficient to significantly induce PGC-1α expression (37). We therefore hypothesized that increasing expression of ERRα may overcome hypoxia-mediated downregulation of PGC-1α mRNA expression. Overexpression of ERRα in cardiomyocytes resulted in a significant increase in ERRα binding to the PGC-1α gene promoter as demonstrated by ChIP (Fig. 4, A and B) and as expected resulted in a significant increase in PGC-1α mRNA expression in normoxic cardiomyocytes (Fig. 4C). Intriguingly, hypoxia-mediated loss of PGC-1α mRNA expression was reversed by ERRα overexpression, rising from 50 to 75% of control normoxic values. Thus, although ERRα does not appear to be involved in recovery of PGC-1α mRNA during reoxygenation, directed overexpression of ERRα is nonetheless capable of significantly attenuating PGC-1α mRNA loss in hypoxia.
Biphasic PGC-1α mRNA expression in response to hypoxia and ischemia. Previous work in H9c2 cells and primary cardiomyocytes indicated that 24-h hypoxia induced PGC-1α expression, in contrast to our results demonstrating that shorter

Fig. 2. Inhibition of PGC-1α expression during hypoxia is due to histone deacetylation. A: chromatin immunoprecipitation (ChIP) of the PGC-1α promoter during hypoxia using an antibody against acetyl-histone H3 (AcH3; K9 residue). IgG, nonspecific immunoglobulin negative control. Results are quantified in B; n = 3. *P < 0.05 vs. normoxia. C: qPCR of PGC-1α expression during hypoxia, with 100 nM TSA or vehicle (DMSO); n = 3. *P < 0.01 vs. normoxia; #P < 0.05 vs. matching DMSO control. D: treatment of cells with 100 nM TSA prevented deacetylation of H3K9 at 12 h of hypoxia compared with vehicle (DMSO)-treated cells as determined by ChIP.

Fig. 3. Recovery of PGC-1α expression during reoxygenation. A: Luciferase assay of PGC-1α promoter transactivation during hypoxia followed by reoxygenation; n = 3. *P < 0.01 vs. normoxia; #P < 0.05 vs. hypoxia without reoxygenation. B: qPCR of PGC-1α expression during hypoxia followed by reoxygenation; n = 3. *P < 0.05 vs. normoxia; #P < 0.05 vs. hypoxia without reoxygenation; &P < 0.05 vs. 12-h reoxygenation. C: qPCR of PGC-1α expression during hypoxia followed by reoxygenation, with 10 μM XCT790 or vehicle (DMSO); n = 3. *P < 0.05 vs. normoxia.
periods of hypoxia caused downregulation (Figs. 1, 2, and 4) (46). We thus extended hypoxia to 24 h and observed a biphasic response of PGC-1α mRNA expression to hypoxia: a significant decrease in expression to ~50% of normoxic control levels at 12 h, followed by a complete rebound to control levels at 24 h (Fig. 5A), suggesting that additional levels of control of expression exist during extended hypoxia.

Zhu et al. (46) reported that upregulation of PGC-1α expression after 24-h hypoxia was due to activation of AMPK, which stimulated PGC-1α expression through an unknown mechanism. In our cell culture conditions, cells were hypoxic but maintained in 18.5 mM glucose. We hypothesized that gradual fuel depletion during hypoxia eventually induced a state of ischemia and resulted in increased AMPK activity (Fig. 5A), supporting our hypothesis that ischemia-induced upregulation of PGC-1α is dependent on AMPK activation.

**Activation of AMPK was confirmed by Western blotting for phosphorylation of AMPKα at threonine 172, normalized to total AMPKα.** In agreement with our model of fuel deprivation during extended hypoxia, AMPKα Thr172 phosphorylation was significantly increased at 24 h but not 12-h hypoxia compared with normoxic control cardiomyocytes (Fig. 5C). This pattern of AMPKα activation corresponded temporally with the increase in PGC-1α mRNA expression (Fig. 5A), supporting our hypothesis that ischemia-induced upregulation of PGC-1α is dependent on AMPK activation.

**Downregulation of PGC-1α expression in an in vivo model of chronic hypoxia.** The above results demonstrate that hypoxia up to 12 h inhibits PGC-1α expression, while continued incubation in an oxygen-poor environment to 24 h results in ischemia and increased PGC-1α expression. Further incubation of primary cardiomyocytes without oxygen beyond 24 h resulted in widespread cell death; thus the chronic effect of oxygen deprivation on PGC-1α expression was unclear. We therefore examined an in vivo rat model of pulmonary hypertension in which animals are maintained in hypobaric hypoxic conditions (10% O₂) for 3 wk (10). These animals experience chronic hypoxia in the absence of ischemia; thus according to our model we would expect generalized inhibition of PGC-1α expression. This was indeed observed: hypoxic rats exhibited significantly decreased right ventricular PGC-1α mRNA and protein expression (Fig. 6A), supporting our hypothesis that decreased oxygen has a negative impact on PGC-1α expression separate from the effect of fuel deprivation. A similar downregulation of PGC-1α protein expression was also observed in the left ventricle of hypoxic animals (Fig. 6A). Although downregulation of left ventricular PGC-1α mRNA did not attain significance in this experiment, expression exhibited a similar trend as in the right ventricle.

Neonatal cardiomyocytes have been well documented to exhibit relative resistance to hypoxia; thus it is possible that the loss of PGC-1α expression in adult hypoxic animals occurred via a different mechanism. To explore this possibility, we isolated adult rat cardiomyocytes and exposed them to 12- or 24-h hypoxia as was performed in neonatal cardiomyocytes. Exposure to 12-h hypoxia significantly downregulated PGC-1α mRNA expression to a similar de-
expression, which on the surface would appear to contradict the results reported here, i.e., that PGC-1α expression was downregulated in hypoxia up to 12 h. In C2C12 myotubes, ischemia relatively quickly induced PGC-1α expression (3). However, ischemia included a reduction in both oxygen and energy substrates, corresponding to our experiment in glucose-free medium, which also demonstrated an increase in PGC-1α expression with extended hypoxia (Fig. 5A). In C2C12 cells, simply reducing nutrient supply in normoxia was sufficient to induce PGC-1α upregulation (3). Since AMPK regulates intracellular signaling pathways and gene expression in response to low fuel supplies, it is possible that AMPK activation was responsible for the induction reported in this previous study.

Relative hypoxia is a hallmark of ischemic heart disease, which also exhibits blockade of the TCA cycle, inhibition of β-oxidation of fatty acids, and a decrease in translocation of ATP from the mitochondria to the cytoplasm (17). Hypoxia attenuates PPARα/RXR-mediated expression of M-CPT I, the rate-limiting enzyme of fatty acid oxidation (23). Since M-CPT I expression is also regulated by PGC-1α, the finding that hypoxia induces HDAC-mediated downregulation of PGC-1α mRNA expression may help to explain these metabolic changes (40). The statistically significant ~20–50% decrease in cardiac PGC-1α protein expression that we observed in hypoxic rats (Fig. 6A) further supports our model that hypoxia in the absence of ischemia results in decreased PGC-1α expression. This result agrees with a previous finding that PGC-1α protein expression is reduced by ~25% in mice exposed to hypoxia (10% O2) for 2 to 4 wk (15). However, this reported decrease was observed in diaphragm muscle, but not gastrocnemius, suggesting that tissue-specific responses to hypoxia exist that may explain a report that PGC-1α expression increases in nonischemic transient brain hypoxia (19). Our results demonstrate that altered PGC-1α expression in the hypoxic heart more closely resembles the change observed in diaphragm muscle compared with other tissues. Loss of PGC-1α expression occurs in decompensated but not compensated cardiac hypertrophy (38). Our animal model exhibits right but not left ventricular hypertrophy and shows no signs of failure at the 3-wk time point employed. Given the reduction in PGC-1α expression in both the left and right ventricles, coupled with alterations of PGC-1α expression in adult cardiomyocytes similar to those observed in neonatal cells, the loss of expression is likely due to hypoxia and not to failure or hypertrophy (Fig. 6).

Our study demonstrates the existence of a previously unappreciated biphasic response of PGC-1α expression to hypoxia.

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**DISCUSSION**

Our data provide novel insight into how hypoxia affects expression of PGC-1α. Previous studies in noncardiac tissues have suggested that hypoxia induces an increase in PGC-1α expression, which as in neonatal cells (~55% of expression in normoxia; Fig. 6B). This was followed, as in neonatal cardiomyocytes, by a significant recovery at 24 h.

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**Fig. 5.** PGC-1α expression exhibits a biphasic response to hypoxia due to histone deacetylation and AMPK activation. A: qPCR of PGC-1α expression in neonatal rat cardiomyocytes with 12- or 24-h hypoxia, with or without 40 μM compound C or vehicle (DMSO). Experiments were carried out in either the presence (+Glc) or absence (~Glc) of 18.5 mM glucose in the cell culture medium; n = 3. *P < 0.05 vs. corresponding normoxia control; #P < 0.05 vs. 24-h hypoxia +Glc; &P < 0.05 vs. 24-h hypoxia ~Glc; SP < 0.05 vs. normoxia +Glc. B: qPCR of PGC-1α expression was carried out as in A in normoxic cardiomyocytes, or in cells exposed to 24-h hypoxia with or without refeeding with glucose-containing (18.5 mM) culture medium after 12-h hypoxia; n = 2–3. *P < 0.05 vs. normoxia. C: phosphorylated AMPKα (Thr172) protein expression was assayed by Western blotting of cardiomyocytes exposed to 12- or 24-h hypoxia or normoxic controls. Results were normalized to total AMPK expression; n = 3. *P < 0.05 vs. normoxia control.
in the heart (Fig. 7). In our proposed model, when oxygen is limiting but glucose is readily available, promoter histone deacetylation results in reduced PGC-1α expression. However, when both oxygen and glucose are limiting, such as during ischemia, AMPK activation results in increased PGC-1α expression (Fig. 5, A and C), an effect that is abrogated by blockade of AMPK or replenishment of fuel (Fig. 5, A and B). One possible mechanism may be via direct AMPK-mediated phosphorylation and activation of PGC-1α protein, which regulates its own gene expression. Our work indicates that it is important for future studies to differentiate the effects of hypoxia (i.e., reduced oxygen) from those of ischemia (i.e., reduced nutrient and oxygen supply) when considering the mechanisms governing the expression of PGC-1α, since low oxygen and low fuel appear to trigger specific and unique responses.

It is possible that other mechanisms contribute to the regulation of PGC-1α expression under conditions of limiting fuel and oxygen. Protein O-GlcNacylation increases in response to cellular stresses such as hypoxia and glucose deprivation, possibly serving as a protective mechanism (8, 45, 47). PGC-1α expression in liver HepG2 cells is increased by manipulations that favor O-GlcNacylation, although whether a similar mechanism exists in cardiomyocytes is unclear (28). Increased O-GlyNacylation in response to glucose deprivation in neonatal rat cardiomyocytes was mediated by AMPK and attenuated by 40 μM compound C, the same concentration employed in the present study to blunt recovery of PGC-1α expression (Fig. 5 A) (47). PGC-1α itself can be O-GlcNacylated; however, the significance of this modification has not yet been determined (20). While it is intriguing to speculate on a possible role of O-GlcNacylation in regulating PGC-1α during glucose deprivation, our finding that PGC-1α expression decreases in hypoxia makes a similar role for O-GlcNacylation in cardiomyocyte hypoxia unlikely.

![Fig. 7. Proposed model of biphasic regulation of PGC-1α expression during hypoxia.](http://ajpcell.physiology.org/)

Fig. 7. Proposed model of biphasic regulation of PGC-1α expression during hypoxia. In early hypoxia, or if glucose and/or ATP are maintained at relatively higher levels, reduced oxygen levels activate HDACs via an unknown mechanism (dotted arrow) to repress PGC-1α gene transcription. This process can be attenuated by HDAC inhibitors such as TSA or by overexpression of ERRα. With longer periods of hypoxia combined with depletion of glucose, i.e., ischemia, ATP levels drop, resulting in activation of AMPK. Through an unknown mechanism (dashed arrow), AMPK stimulates transactivation of the PGC-1α gene. This process can be attenuated by the AMPK inhibitor compound C or by supplementation with exogenous glucose. Shaded shapes represent the basal transcriptional machinery.

![Fig. 6. PGC-1α expression is downregulated in an in vivo model of chronic hypoxia.](http://ajpcell.physiology.org/)

Fig. 6. PGC-1α expression is downregulated in an in vivo model of chronic hypoxia. A: PGC-1α mRNA and protein expression was assayed by qPCR or Western blotting, respectively, in right (RV) or left (LV) cardiac ventricles from rats following 3 wk of hypobaric hypoxia (10% O2); n = 5–8. *P < 0.05 vs. normoxia. B: isolated adult rat cardiomyocytes were normoxic, or exposed to 12 or 24-h hypoxia, then assayed for PGC-1α mRNA expression by qPCR; n = 2–3. *P < 0.05 vs. normoxia; #P < 0.05 vs. 12-h hypoxia.
Our findings may help explain in part the therapeutic benefits observed with class I and pan-HDAC inhibitors in treatment of cardiac disease models such as pressure overload in which PGC-1α expression typically decreases: general HDAC inhibition would be expected to spare PGC-1α expression (Fig. 2C), which is critical since loss of PGC-1α expression promotes pressure overload-induced heart failure (6, 14, 18, 26, 32). We observed a similar effect of TSA on hypoxia-induced loss of ERα expression (data not shown), which may be important considering the significant overlap of ERα and PGC-1α target genes and the similar negative impact of deletion of these genes on cardiac function (4, 6, 21, 32, 39). Since ERα overexpression was sufficient to rescue the hypoxia-mediated loss of PGC-1α expression (Fig. 4C), TSA may exert beneficial effects on PGC-1α expression both directly and indirectly via ERα.

The inhibition of prohypertrophic class I HDACs concomitant with promotion of the antihypertrophic activity of class II HDACs may be beneficial for treatment of cardiac diseases (7). However, we previously demonstrated that the class IIa HDAC5 potently inhibits PGC-1α expression and have suggested that HDAC5 and MEF2 transcription factors coordinately regulate the expression of prohypertrophic genes along with genes involved in energy metabolism via control of PGC-1α expression (12, 13). Therapeutically manipulating class IIa HDAC activity may thus be problematic. The pan-HDAC inhibitor TSA completely attenuated the loss of both PGC-1α and ERα expression induced by hypoxia (Fig. 2, C and D). Pharmacologic approaches that induce general HDAC repression while augmenting ERα activity and/or expression may thus provide novel approaches to antihypertrophic therapy with minimal negative impact on energy metabolic gene expression.

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