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Hypoxia *in Vivo* Decreases Peroxisome Proliferator-Activated Receptor α-Regulated Gene Expression in Rat Heart

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We tested the hypothesis that hypoxia decreases PPARα-regulated gene expression in heart muscle *in vivo*. In two rat models of systemic hypoxia (cobalt chloride treatment and iso-volemic hemodilution), transcript levels of PPARα and PPARα-regulated genes (pyruvate dehydrogenase kinase 4 (PDK4), muscle carnitine palmitoyltransferase-I (mCPT-I), and malonyl-CoA decarboxylase (MCD)) were measured using real-time quantitative RT-PCR. Data were normalized to the housekeeping gene β-actin. Atrial natriuretic factor (ANF) and pyruvate dehydrogenase kinase 2 (PDK2), which are not regulated by PPARα, served as controls. $CoCl_2$ treatment decreased PPARα, PDK4, mCPT-I, and MCD mRNA levels. Iso-volemic anemia also caused a significant decrease in PPARα, PDK4, and MCD mRNA levels. Transcript levels of mCPT-I showed a slight, but not significant decrease ($P = 0.08$). Gene expression of β-actin, ANF, and PDK2 did not change with either $CoCl_2$ treatment nor with anemia. Myocardial PPARα-regulated gene expression is decreased in two models of hypoxia *in vivo*. These results suggest a transcriptional mechanism for decreased fatty oxidation and increased reliance of the heart for glucose during hypoxia.

Key Words: **cobalt chloride; iso-volemic hemodilution; quantitative RT-PCR.**

Myocardial hypoxia induces a switch in energy substrate preference from predominant oxidation of fatty acids to increased glucose utilization (1). Hypoxia inducible factor 1α (HIF-1α) has been identified as one transcriptional mechanism for the increased reliance on glucose as an energy substrate during hypoxia through induction of glucose transporter 1 and various glycolytic enzymes (2). In contrast, little is known about the transcriptional mechanism responsible for the down-regulation of mitochondrial oxidation during hypoxia. The nuclear receptor peroxisome proliferator-activated receptor α (PPARα) controls the expression of several key regulators of glucose (e.g., pyruvate dehydrogenase kinase 4 (PDK4)) and fatty acid oxidation (e.g., malonyl CoA decarboxylase (MCD), and muscle carnitine palmitoyl transferase-I (mCPT-I)) (3–5). Evidence suggests that PPARα plays a key role in the metabolic adaptation of the heart to pressure overload (6). In addition, a recent study has shown that the DNA-binding activity of the PPARα/RXR heterodimer is decreased during hypoxia in cell culture (7).

We therefore decided to test whether PPARα and PPARα regulated gene expression decrease in response to hypoxia *in vivo*. We measured gene expression of PPARα, PDK4, MCD, and mCPT-I in the rat heart of animals exposed to two different models of hypoxia. The results suggest that down-regulation of PPARα may play a role in substrate switching observed in the heart in response to chronic hypoxia.

METHODS

In Vivo Models of Hypoxia

Cobalt chloride treatment. Male Sprague–Dawley rats (250 g, Harlan, Indianapolis, IN) received $CoCl_2$ in their drinking water (2 mM solution) for 10 days as described by Badr *et al.* (8). Based on the daily fluid intake each animal received approximately 60 μmol $CoCl_2$ per day. There was no difference in water or food intake between the control and experimental group (data not shown). Body weight and heart weight to body weight ratio also did not differ between the two groups after 10 days of $CoCl_2$ treatment (data not shown). After the treatment period animals were anaesthetized (pentobarbital, 100 mg/kg body weight, intra-peritoneal), hearts were rapidly removed, freeze clamped, and stored at −80°C.

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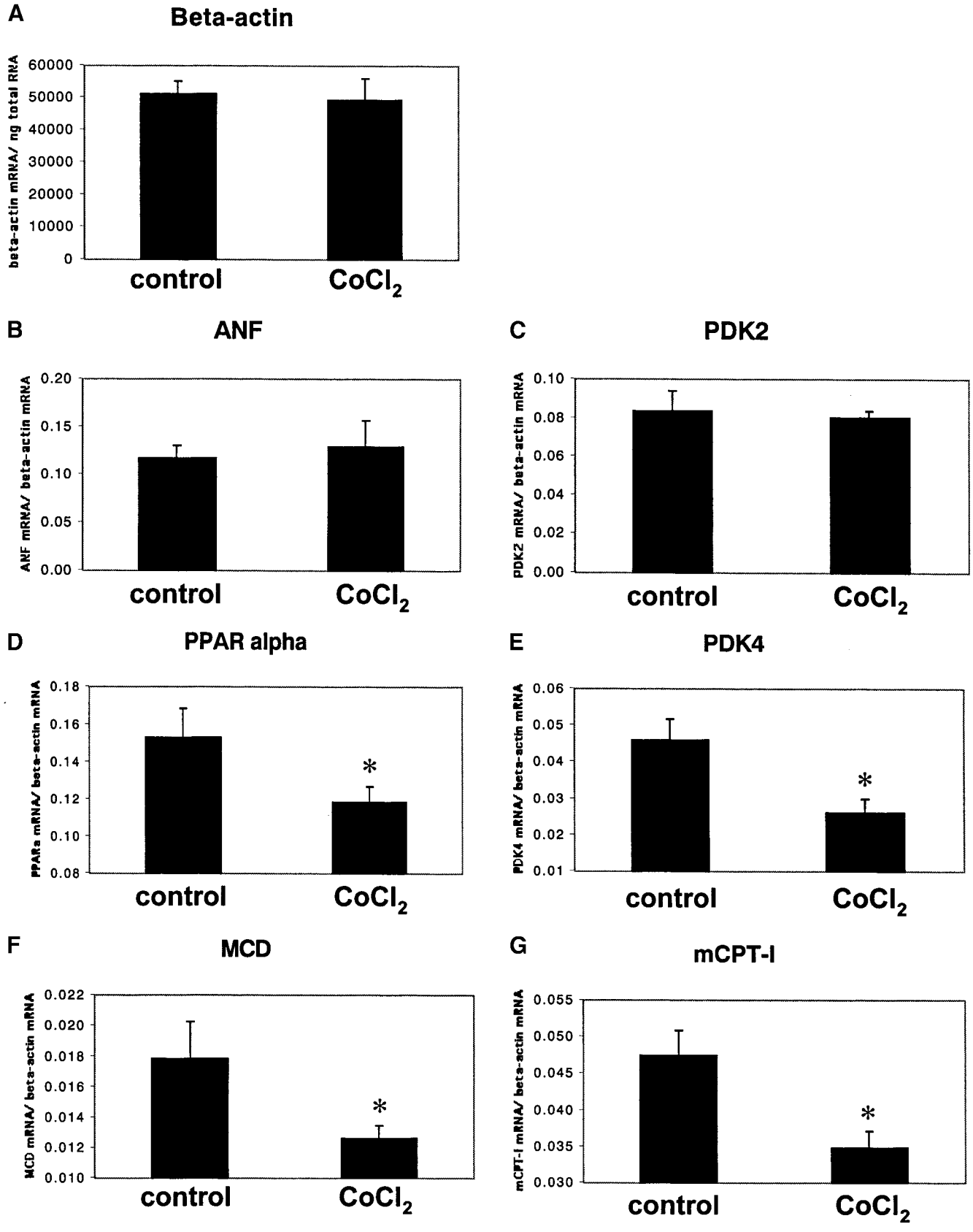

FIG. 1. PPARα and PPARα regulated gene expression decrease with $CoCl_2$ treatment, while β-actin, PDK2, and ANF do not change ($^*P < 0.05$).

Iso-volemic hemodilution. Iso-volemic hemodilution was performed in male Sprague–Dawley rats (300 g, Harlan, Indianapolis, IN). A right external jugular vein catheter was implanted as previously described (9). Briefly, the right external jugular vein was exposed and gently cleaned by blunt dissection. The vein was semi-transected with fine scissors between two ligatures. A catheter (silicon tube, Allied Biomedical, Paso Robles, CA), attached to a Tygon-Tube (Cole-Parmer Instruments Company, London, Great Britain) filled with a heparin-saline solution (20 units heparin/ml normal saline) was inserted into the vein and the blunted tip was advanced by 2–3 cm toward the heart. The catheter was sutured to the soft tissue and skin incisions were closed.

Three days after the initial surgery, animals were restrained, and 1 ml of blood was withdrawn over a period of 1 min into a syringe containing 10 μl of heparin (10,000 U/ml). The blood was centrifuged (400 RPMI for 1 min), plasma was transferred into a new tube and mixed with prewarmed (37°C) normal saline to a total volume of 1 ml. The plasma/saline solution was then slowly injected through the catheter over a period of one minute. In the control group blood was drawn and slowly reinjected. This procedure was repeated every hour for 9 h to complete a total blood exchange of 10 ml. Hematocrit and hemoglobin were measured before the procedure (baseline) and two days after the hemodilution by using Vet Test (Idexx, Westbrook, ME). Two days after the bleeding animals were anaesthetized (pentobarbital, 100 mg/kg body weight, intra-peritoneal), and hearts were rapidly removed, freeze clamped, and stored at −80°C.

Measurement of gene transcripts. RNA was extracted by standard methods (10), and analyzed by reverse transcription followed by real-time quantitative PCR for β-actin, ANF, PDK2, PDK4, PPARα, MCD, and mCPT-I. The methodology of quantitative PCR has been described in detail previously (11). The nucleotide sequences for probes as well as forward and reverse primers of the rat transcript have been published previously (4, 12, 13). The transcript for the constitutive gene product β-actin was used as a reporter gene for data normalization. Internal RNA standards were prepared using the T7 RNA polymerase method (Ambion, Austin, TX) (11).

Statistical analysis. Data are expressed as means ± SEM. Differences between the control and the experimental group were calculated by Student's *t*-test for independent variables. A value of $P < 0.05$ was considered as significant.

RESULTS

Cobalt Chloride Treatment

Figure 1A shows transcript levels of the housekeeping gene β-actin. As negative controls we also measured transcript levels of a heart failure marker (ANF) and of a metabolic gene (PDK2), which are not believed to be regulated by PPARα (Figs. 1B and 1C). Figures 1D–1G show mRNA levels of PPARα and the PPARα-regulated genes PDK4, MCD, and mCPT-I. Transcript levels of β-actin, ANF and PDK2 were unaffected by $CoCl_2$ treatment. In contrast, the mRNA levels of PPARα, PDK4, MCD, and mCPT-I all decreased with $CoCl_2$ treatment.

Iso-volemic Hemodilution

The hematocrit was decreased by approximately 50% on the second day after the bleeding procedure (Fig. 2). This fall in hematocrit was accompanied by a similar fall in hemoglobin (data not shown).

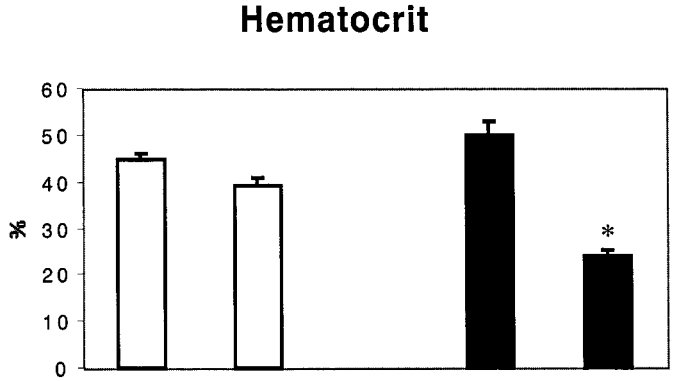

FIG. 2. Hematocrit level are decreased 3 days after iso-volemic hemodilution (*$P < 0.05$).

Figures 3A–3C show that β-actin, ANF and PDK2 were not affected by 2 days of anemia. In contrast, transcript levels of PPARα, PDK4, and MCD significantly decreased in the anemia group (Figs. 3D–3F). Messenger RNA levels of mCPT-I showed a slight, but not significant decrease ($P = 0.08$, Fig. 3G).

DISCUSSION

The heart alters energy substrate preference in response to both acute and chronic environmental stimuli. Fatty acids are the primary energy substrate in the normal adult heart (14). During cardiac hypertrophy and during hypoxia the heart increases its reliance on carbohydrate utilization and decreases long chain fatty acyl-CoA oxidation (6). The transcriptional mechanisms underlying this switch in energy substrate preference are not well understood (15). The present study shows that the expression of the nuclear receptor PPARα and PPARα-regulated genes are decreased in two different *in vivo* models of hypoxia and suggests a potential transcriptional mechanism for the decrease in long chain fatty acyl-CoA oxidation during hypoxia.

Energy Substrate Metabolism during Hypoxia

During hypoxia the rate of ATP production from oxidative phosphorylation of fatty acid derived substrates is decreased to a greater extent than the rate of glucose oxidation, while rates of glycolysis are increased (16). Acutely, both glucose transporters 1 and 4 translocate to the cell surface, glycogenolysis is stimulated, and the activities of hexokinase and phosphofructokinase increase during hypoxia, due in part to an increase in the AMP/ATP and Cr/PCr ratios, and the subsequent activation of AMP-activated protein kinase. As pyruvate accumulates, lactate is produced, allowing the regeneration NAD^+, and therefore maintenance of glycolytic flux (17). Under these conditions activated fatty acids are diverted from β-oxidation into deposition as tissue lipids (18). During oxygen-

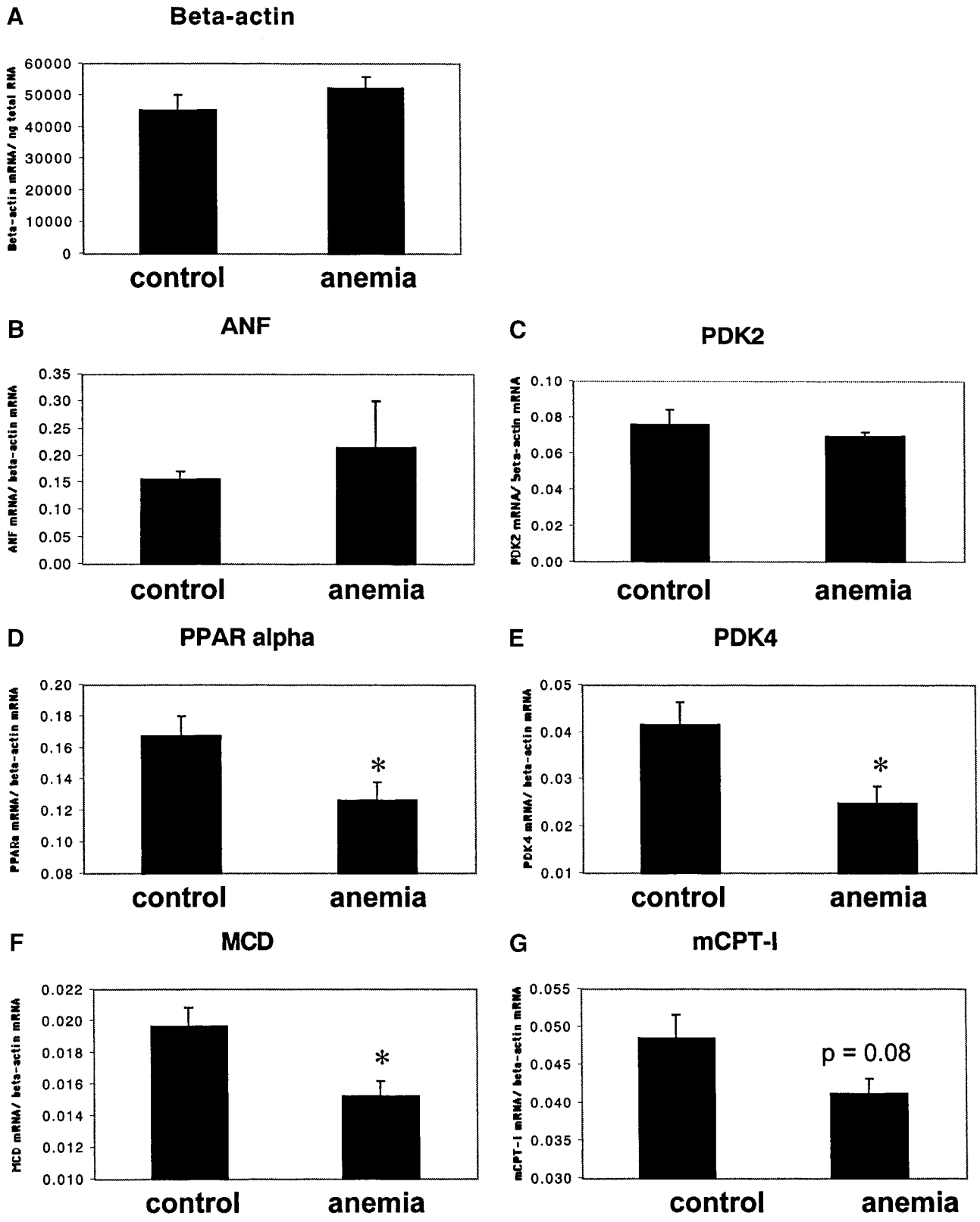

FIG. 3. Iso-volemic hemodilution induces a decrease in PPARα, PDK4, and MCD expression, while transcript levels of mCPT-I show the trend to decrease. β-actin, ANF, and PDK2 do not change ($^*P < 0.05$).

deficient states increased reliance on glucose oxidation at the expense of fatty acid oxidation increases efficiency (i.e., number of ATP molecules per mole O_2), while a better coupling of glycolysis and pyruvate oxidation will lower proton formation. Thus, metabolic adaptations during hypoxia are geared to preserve cellular function and integrity. If those hypoxic conditions persist, transcriptional adaptation occurs (19). Work focusing on HIF-1α has shown that this transcription factor is involved in the induction of Glut1 and several glycolytic enzymes, thus maintaining reliance on glycolysis-derived ATP (2). Less is understood about the transcriptional mechanisms involved in the oxidative metabolism of the heart during hypoxia.

In Vivo Models of Hypoxia

Initial observations in isolated neonatal cardiomyocytes found that hypoxia decreased PPARα DNA-binding activity (7). We have now found consistent results in two different *in vivo* models of hypoxia. In the first model rats received $CoCl_2$ in their drinking water. Cobalt is a transition metal, that replaces iron in heme proteins. In contrast to iron, cobalt does not bind oxygen when incorporated into protoporphyrin (20). Previous studies have suggested a putative oxygen sensor in the cell membrane that contains a heme protein (20). When iron is replaced by cobalt, this oxygen sensor signals to the cell that there is a state of oxygen deficiency despite the normal oxygen levels. In a second model of hypoxia we induced iso-volemic hemodilution by bleeding, followed by retransfusion of a plasma/saline solution. Anemia is characterized by a reduced oxygen binding capacity, resulting in lower tissue oxygenation. Previous studies have shown that both models induce gene expression of markers of hypoxia (e.g., erythropoietin) in various cell types (21).

PPARα Is a Metabolic Switch in the Heart

The nuclear receptor PPARα regulates the transcription of key regulators of pyruvate and long chain fatty acyl-CoA (LCFA) oxidation. Pyruvate dehydrogenase kinase (PDK) inhibits glucose oxidation by phosphorylation of the pyruvate dehydrogenase complex. Several isoforms of PDK are described in the heart. PDK1 and PDK2 are considered as constitutive isoforms, where as PDK4 expression is inducible through PPARα activation (normally in response to an increase in fatty acid availability). A previous report has shown that transcript levels of PDK4 correlate positively with inhibition of glucose oxidation, suggesting that PDK4 activity is regulated at least partially on the transcriptional level (22).

PPARα controls several genes encoding for proteins in LCFA oxidation, including CPT-I, which regulates the rate-limiting step of LCFA transport across the inner mitochondrial membrane. In the heart two isoforms of CPT-I exist (liver and muscle) (23). The liver isoform is of low abundance and is expressed relatively constitutively, while the muscle isoform is highly abundant and inducible in the heart (23). CPT-I activity is allosterically inhibited by malonyl-CoA. Malonyl-CoA levels are determined by the rates of synthesis (by acetyl-CoA carboxylase) and degradation (by malonyl-CoA decarboxylase (MCD)). We have previously shown that MCD expression is regulated by PPARα and that MCD transcript levels often change in parallel with fatty acid oxidation rates (4). Thus, PPARα appears to regulate the energy substrate switch from glucose to LCFA oxidation at the transcriptional level.

Hypoxia Decreases PPARα-Regulated Gene Expression

Hypoxia regulates the activity of numerous transcription factors including AP-1, HIF-1α, Sp1, and NF-kappaB (19). A recent *in vitro* study showed that hypoxia reduces PPARα/RXR DNA-binding activity and decreases mCPT-I gene expression (7). We found that in addition to mCPT-I expression, two other PPARα-regulated genes (PDK4, MCD) show similar responses in the current *in vivo* models of hypoxia. Furthermore, gene expression of PPARα itself decreased in both models. Others have shown that PPARα gene expression correlates with PPARα protein levels and PPARα DNA-binding activities (13, 24). These findings suggest that the decrease in PPARα-regulated gene expression observed in the present study is likely due to decreased PPARα expression and activity. The present results may explain the metabolic adaptation of the heart in response to chronic hypoxia.

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