Dynamic changes of gene expression in hypoxia-induced right ventricular hypertrophy

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Dynamic changes of gene expression in hypoxia-induced right ventricular hypertrophy. Am J Physiol Heart Circ Physiol 286: H1185–H1192, 2004. First published November 20, 2003; 10.1152/ajpheart.00916.2003.—Hypobaric hypoxia induces right ventricular hypertrophy. The relative contribution of pulmonary hypertension, decreased arterial oxygen and neuroendocrine stimulation to the transcriptional profile of hypoxia-induced right ventricular hypertrophy is unknown. Whereas both ventricles are exposed to hypoxia and neuroendocrine stimulation, only the right ventricle is exposed to increased load. We postulated that right ventricular hypertrophy would reactivate the fetal gene transcriptional profile in response to increased load. We measured the expression of candidate genes in the right ventricle of rats exposed to hypobaric hypoxia (11% O2) and compared the results with the left ventricle. Hypoxia induced right ventricular hypertrophy without fibrosis. In the right ventricle only, atrial natriuretic factor transcript levels progressively increased starting at day 7. Metabolic genes were differentially regulated, suggesting a substrate switch from fatty acids to glucose during early hypoxia and a switch back to fatty acids by day 14. There was also a switch in myosin iso-gene expression and a downregulation of sarcoplasmic/endoplasmic ATPase 2a during early hypoxia, whereas later, both myosin isoforms and SERCA2a were upregulated. When the right and left ventricle were compared, the transcript levels of all genes, except for myosin isoforms and pyruvate dehydrogenase kinase-4, differed dynamically, this model is associated with increased pulmonary arterial pressure without alteration in systemic blood pressure (18). We measured the transcript levels of metabolic, sarcomeric, calcium regulating, and fetal genes in both ventricles. Although both ventricles were exposed to hypoxia and neuroendocrine stimulation, only the right ventricle was exposed to increased load. Therefore, we compared the transcriptional profile of the right and left ventricle to determine the effect of load on gene expression of the hypertrophied right ventricle. This approach had been utilized previously to determine the contribution of load on gene expression (14, 16).

Our findings demonstrate that there are dynamic changes in gene expression of the rat heart with hypoxia-induced RV hypertrophy that are characterized by an early reactivation of the fetal transcriptional profile and a late reversion back to an adult pattern. Furthermore, myosin iso-gene and pyruvate dehydrogenase kinase-4 (PDK-4) transcript levels are not affected by load, suggesting that either hypoxia itself or neuroendocrine stimulation primarily regulate these genes.

METHODS

In vivo exposure to hypoxia. Six-week-old male Wistar rats were housed inside a Lexan chamber (SciTech; Cape Town, South Africa), as described previously (19). Rats were exposed to a hypobaric hypoxic stimulus (45 kPa, 11% ambient O2) for a period of 2, 7, or 14 days, after which they were euthanized with CO2. When specified, the rats died after 12 or 24 h of hypobaric hypoxia exposure to evaluate earlier time points. To prevent any bias due to diurnal variations, the animals were euthanized between 2 and 4 PM. Intact hearts were removed and RV and left ventricular (LV) tissues were dissected out, weighed, and stored at −80°C for subsequent molecular analyses. Age- and sex-matched normoxic controls were used for all experiments. The chamber was opened every 48 h for no >20 min to replace bedding, clean cages, and provide fresh food and water. The University of Cape Town’s Animal Research Ethics Committee approved all

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animal experiments, and the investigation conforms to the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Publication No. 85-23, Revised 1996).

Determination of plasma glucose, nonesterified fatty acid levels, and hematocrit. At the end of each experimental time point, blood was collected by cardiac puncture from both hypoxic and normoxic rats. Blood collection was routinely performed between 2 and 6 PM. The samples were centrifuged in a microfuge at 3,500 rpm for 10 min, and the supernatant was used to determine plasma glucose and nonesterified fatty acid (NEFA) levels. Plasma NEFA levels were spectrophotometrically determined using a commercially available kit (Roche: Basel, Switzerland). Plasma glucose levels were measured with a Beckman glucose analyzer (Beckman Instruments; Fullerton, CA). Hematocrit was determined by collecting blood in heparinized capillary tubes and centrifuging it at 12,000 rpm for 3 min in a hemofuge.

Gene expression. The method for RNA extraction and for quantitative RT-PCR has been described previously (5). The nucleotide sequences for primers and probes also have been previously published (5, 32). The transcript for the constitutive gene 18S was used as a housekeeping gene for data normalization. Internal standards were prepared using the T7 RNA polymerase method (Ambion; Austin, TX).

Histology. Heart tissue (~1 cm) from normoxic and hypoxic animals was placed in histology cassettes and fixed using 10% formalin for 4 h. Samples were subsequently left in 70% ethanol and processed by dehydrating the tissue through graded alcohols. Tissues were then embedded in paraffin wax, sectioned at 1 μm thickness, and stained with hematoxylin and eosin (HE). Sirius red was used to visualize collagen deposits (fibrosis) as described before (1). Photomicrographs of HE-stained (×40 magnification) and Sirius red (×10 magnification)-stained sections were taken on a Zeiss Axioshot with the use of a Leaf Microlumina digital camera. Myocyte diameter was determined by measuring the short axis of the myocytes from 30 images of HE-stained sections using Image Pro Plus software. A total of 20 images of Sirius red-stained sections were analyzed with Image Pro Plus software using color-cube-based selection criteria to ensure that only Sirius red-stained regions were counted. The area of Sirius red staining from all fields per photomicrograph was averaged with background subtraction (nontissue regions).

Statistical analysis. Data are expressed as means ± SE. Differences between the groups were calculated by one-way ANOVA, followed by a Bonferroni test. A value of \( P < 0.05 \) was considered significant.

RESULTS

Compensatory adaptation to chronic hypoxia. Rats exposed to hypoxia demonstrated a 43% increase in hematocrit at day 7, with a further increase to 56% above baseline by day 14 (Fig. 1). The RV-to-LV weight ratio increased by 22% at 2 days, and then increased by 53% at day 7, and remained stable thereafter (Fig. 1). Thus hypobaric hypoxia induced an early trophic response in the right ventricle that was complete at day 7. Microscopic analysis of tissue from right ventricle demonstrated an increase in myocyte size without fibrosis (Fig. 2). Serum blood glucose levels did not change with hypoxia; however, NEFA levels decreased by 42% (data not shown).

Atrial natriuretic factor expression. Atrial natriuretic factor (ANF) was induced rapidly in response to pressure overload (8). Not surprisingly, ANF expression was increased at day 7 and continued to rise at day 14 of hypoxia exposure in the right ventricle (Fig. 3). There was no change in ANF transcript levels in the left ventricle. These results suggest that load-induced changes in gene expression in the right ventricle occurred between days 2 and 7.

Genes regulating fatty acid metabolism. Peroxisome proliferator-activated receptor-α (PPARα), a nuclear receptor that orchestrates the expression of several metabolic genes, is considered to be a key regulator of substrate switching in the hypertrophied heart (2). We (19, 23) have shown that hypoxia downregulates the PPARα gene and protein expression in the rodent heart. PPARα and medium-chain acyl-CoA dehydrogenase (MCAD), a PPARα-regulated gene, were downregulated at day 7 of hypoxia (Fig. 4). In contrast, transcript levels of both PPARα and MCAD were dramatically increased at day 14 of hypoxia. There was no change in PPARα expression in the left ventricle at any time point, suggesting that expression of both genes was regulated by load. MCAD transcript levels were slightly increased in the left ventricle at day 14. Our findings suggest that the right ventricle initially switches substrate preference from fatty acid to glucose oxidation but eventually switches back to fatty acid utilization.

Genes regulating glucose metabolism. The rate-limiting step for exogenous glucose utilization is its transport into the cardiomyocyte through specific carrier-mediated transporters referred to as GLUT1 and GLUT4 (6). GLUT4 is the main isoform found in the normal adult heart, whereas GLUT1 is primarily expressed in the fetus (25). PDK-4 is a key regulator of glucose and lactate oxidation through inhibitory phosphorylation of pyruvate dehydrogenase (26). Although there was no significant difference in GLUT1 and GLUT4 expression at days 2 and 7, GLUT1 transcript levels trended downward, whereas GLUT4 expression tended to increase. By day 14, GLUT1 expression was significantly downregulated and GLUT4 transcript levels were increased (Fig. 5), suggesting an accentuation of the adult gene expression pattern. PDK-4 transcript levels were dramatically decreased at day 2, implying an early increase in glucose oxidation. However, PDK-4 expression returned to baseline by day 7 and then increased by nearly 2.5-fold compared with normoxic right ventricles at day 14.

Fig. 1. Compensatory adaptation to chronic hypoxia (n = 5 at each time point). RV/LV, right ventricular-to-left ventricular weight ratio (B). Hematocrit (A) begins to rise at 7 days of hypobaric hypoxia exposure (* \( P < 0.001 \), compared with baseline) and continues to rise at 14 days of hypoxic exposure (** \( P < 0.001 \), compared with 7 days). The weight of the right ventricle increases up to 7 days but then stabilizes (B: * \( P < 0.05 \), compared with baseline).
14. This fluctuation suggests an inhibition of glucose oxidation at day 14. PDK-4 expression in the left ventricle paralleled right ventricle expression, indicating that this gene is not regulated by load. GLUT1 and GLUT4 expression in the left ventricle did not change significantly with hypobaric hypoxia, indicating that changes observed in the right ventricle were likely load dependent.

Sarcomeric genes. Myosin heavy chain (MHC), the main component of myosin, exists in two distinct isoforms (21). Myosin composed of predominately MHC-β isoform has a decreased ATPase activity compared with MHC-α, which results in decreased contractile velocity but greater economy in force generation (11). At day 2 of hypoxia, there was a downregulation of MHC-α expression in the right ventricle that returned to baseline at day 7 and was dramatically increased at day 14 (Fig. 6). MHC-β transcript levels were increased by over 20-fold at 2 days. Unexpectedly, MHC-β expression was downregulated by 3.5-fold compared with baseline at 7 days. By day 14, transcript levels of MHC-β again increased by nearly ninefold compared with baseline. At all time points, MHC-α and MHC-β transcript levels in the left ventricle were similar to those levels in the right ventricle, suggesting the observed changes were independent of load.

Calcium regulating genes. The sarcoplasmic/endoplasmic reticulum ATPase 2a protein (SERCA2a) maintains and regulates the Ca$^{2+}$ content of the sarcoplasmic reticulum (3). Ca$^{2+}$ released from the sarcoplasmic reticulum is the major source of intracellular calcium during excitation-contraction coupling. Thus SERCA2a activity is important in regulation of cardiac contractility (3). SERCA2a transcript levels decreased in both the right and left ventricle at day 2, suggesting load-independent regulation (Fig. 6). At day 7, there was a continued decrease in SERCA2a expression in the right ventricle; however, at day 14, transcript levels increased by 6.5-fold. These changes did not occur in the left ventricle.

**DISCUSSION**

The purpose of this investigation is to elucidate the transcriptional profile of hypoxia-induced RV hypertrophy. We
examined the right ventricle at days 2, 7, and 14 of hypobaric hypoxia exposure to delineate early and late changes in gene expression. We compared the right and left ventricle to determine the effect of load on gene expression because only the right ventricle is exposed to increased load (e.g., pulmonary hypertension).

The main finding of this study is that there are dynamic changes in gene expression in hypoxia-induced RV hypertrophy characterized by an early reactivation of the fetal gene program that reverts back to an adult pattern. Furthermore, the transcript levels of all genes, except for myosin isoforms and PDK-4, differ dramatically between ventricles, suggesting that all of these genes are regulated by load.

Metabolic genes. Although the heart primarily oxidizes fatty acids under basal conditions, it has the capability to utilize a variety of substrates (9, 28). The heart can switch substrate preference in response to multiple stimuli, which can include substrate availability, coronary blood flow, circulating hormones, and workload (27). This allows the heart to “choose” the right substrate at the right moment. For example, the heart responds to acute increase in workload by oxidizing glycogen, lactate, and glucose (10). This ability to alter substrate preference is a phenomenon we refer to as metabolic flexibility (29).

In contrast, heart failure is associated with a persistent substrate switch to glucose (4, 24). In other words, there is a loss of metabolic flexibility. We believe this loss of metabolic flexibility is an early sign of a maladaptive transcriptional profile that contributes to cardiac dysfunction in the failing heart (29).

The initial response of the right ventricle to chronic hypoxia is a downregulation of PDK-4 expression after 2 days. Because PDK-4 inhibits glucose oxidation, this response implies an early switch in substrate utilization from fatty acids to glucose. The same expression pattern is also observed in the left ventricle, indicating that this early substrate switch is independent of load. In any case, ANF expression begins to increase at day 7, suggesting that pressure-induced changes in gene expression are not observed until this time point. Although we cannot differentiate between the effects of hypoxia and neuroendocrine stimulation, we have previously shown that PDK-4 is downregulated in other models of hypoxia (23). The steady rise in PDK-4 expression in the hypoxic right ventricle.
also parallels the rise hematocrit levels. We speculate that as compensatory mechanisms restore normoxia (e.g., rise in hematocrit levels) PDK-4 expression also increases.

It is well known that pressure overload decreases PPARα expression in the rat left ventricle (2). In the present study, we also observe a decrease in PPARα and MCAD expression at day 7 that appears to be regulated by load. However, this is in contrast to previous studies (12), in which PPARα expression is downregulated by hypoxia. We found that hypoxia decreases PPARα expression at 12 h in both the right and left ventricle, and expression returns to baseline by 24 h (P. Razeghi, S. Sharma, E. Essop, and H. Taegtmeyer, unpublished observations). Thus PPARα expression and activity appear to be regulated by both hypoxia (early) and load (late). Interestingly, the downregulation of PPARα and MCAD at day 7 is completely reversed and actually upregulated by day 14. We postulate that as the trophic adaptation of the heart compensates for increased load, the right ventricle switches back to fatty acid oxidation. MCAD transcript levels are similarly affected in the left ventricle at day 14 but to a lesser extent. We speculate that this moderate increase in MCAD expression reflects normalization of hypoxia by compensatory mechanisms (e.g., erythrocytosis) supporting the notion that PPARα is regulated by both hypoxia and load.

Glucose is a more efficient fuel in terms of oxygen consumption for ATP generation than fatty acids (27). Thus it is not surprising that under conditions of decreased oxygen delivery, the heart switches to the more efficient fuel. While compensatory mechanisms restore normoxia and trophic adaptation compensates for increased load in the right ventricle, there is restoration of a normal metabolic transcriptional profile in the right ventricle and subsequently a switch back from glucose to fatty acid oxidation. Thus in hypoxia-induced RV hypertrophy metabolic flexibility is maintained.

**Myosin iso-genes.** As mentioned earlier, MHC-β has decreased contractile velocity but conserves more ATP per contraction than MHC-α. Hypoxia increases MHC-β iso-gene expression while decreasing MHC-α expression in the left ventricle and in vitro (22). Myosin iso-gene switching in response to hypoxia is thought to represent an adaptive mechanism, similar to substrate switching, which conserves myocardial oxygen. The dramatic load-independent upregulation of MHC-β and the downregulation of MHC-α at day 2 support this concept. Furthermore, MHC-α expression in both right and left ventricle steadily increases with time, paralleling the rise in hematocrit. This suggests that compensatory mechanisms that restore normoxia also restore MHC-α expression.

The decrease in MHC-β transcript levels at day 7 is surprising. This sudden decrease in MHC-β transcript levels affects both ventricles and thus cannot be attributed to pressure overload of the right ventricle. Compensatory mechanisms do not

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**Fig. 5.** Genes regulating glucose metabolism (n = 5 at each time point). GLUT1 expression trends downward and is significantly decreased by 14 days. GLUT4 transcript levels steadily increase until significantly elevated at day 14. Pyruvate dehydrogenase-4 (PDK-4) expression is downregulated at 2 days but steadily increases until significantly upregulated by day 14. GLUT1 and GLUT4 transcript levels do not change in the left ventricle, whereas PDK-4 expression in the left ventricle parallels RV expression (*P < 0.05). A: right ventricle; B: left ventricle.
restore normoxia by day 7, as evidenced by the continued rise in hematocrit. Furthermore, it is unlikely that there was any sudden change in arterial oxygen content at day 7 that resolved by day 14. Thus arterial oxygen content is not likely to be affecting MHC-β expression at day 7. We speculate that there must be some neuroendocrine factor (e.g., sympathetic stimulation) that is affecting MHC-β expression in the right ventricle at day 7 of hypobaric hypoxia. This neuroendocrine stimulation either eventually diminishes or the right ventricle adapts to it because MHC-β expression is upregulated once again by day 14.

SERCA2a. SERCA2a transcript levels are downregulated in fetal and failing heart (24), and this downregulation is thought to contribute to contractile dysfunction in the failing heart (20). In response to hypobaric hypoxia, the initial gene expression profile is also characterized by a small decrease in SERCA2a transcript levels, suggesting slight impairment of cardiac contractility. Recent studies (14) have also shown that pressure overload of the right ventricle results in downregulation of SERCA2a expression in association with impaired calcium handling and contractile function. However, we demonstrated a downregulation of SERCA2a transcript levels at day 2 of hypoxia in both the right and left ventricle, indicating load-independent regulation of SERCA2a at this time point. This downregulation of SERCA2a may represent an ATP conserving adaptation in the face of decreased arterial oxygenation. While SERCA2a expression was restored in the left ventricle by day 7, transcript levels in the right ventricle remained depressed before they dramatically increased at day 14. We speculate that early changes in SERCA2a expression are regulated by decreased arterial oxygen content and therefore affect both ventricles. While hypoxia is normalized by compensatory mechanisms, the expression of SERCA2a returns to normal in the left ventricle. However, the right ventricle is still exposed to increased load secondary to pulmonary hypertension resulting in persistent decreased SERCA2a transcript levels at day 7. While trophic adaptation of the right ventricle compensates for increased load (i.e., day 14), SERCA2a expression is upregulated to maintain contractile performance.

Adaptive versus maladaptive transcriptional profile. Hypertrophy of the left ventricle is frequently classified as adaptive or maladaptive; this is based, in part, on the transcriptional profile induced by the trophic stimulus (13). Maladaptive gene expression is indicated by induction of the fetal transcriptional profile, where an adaptive transcriptional profile demonstrates an adult gene expression pattern (7). We (5) have shown that atrophy of the heart also induces fetal gene expression. Thus the fetal gene expression profile is not a characteristic specific to hypertrophy. In the present study, we demonstrate fluctuations in the gene expression profile without a change in the

Fig. 6. Sarcomeric and calcium regulating genes (n = 5 at each time point). Myosin heavy chain-α (MHC-α) transcript levels are decreased at day 2 and steadily increase until significantly upregulated by day 14. MHC-β expression is initially high at day 2 and then markedly downregulated at day 7. By day 14, MHC-β expression is once again elevated. The same expression pattern of MHC-α and MHC-β is seen in the left ventricle (B). Sarcoplasmic/endoplasmic ATPase 2a (SERCA2a) transcript levels are decreased at days 2 and 7 in the right ventricle (A), whereas in the left ventricle, SERCA2a expression is only mildly decreased at day 2 (P = 0.06). At day 14, RV SERCA2a expression is markedly upregulated (*P < 0.05, **P < 0.001).
trophic response of the heart to chronic hypoxia. Our results support the concept of dissociation between the trophic and the transcriptional adaptation of the heart to stress. Thus to classify hypertrophy as adaptive or maladaptive by a static measurement in gene expression profile may be misleading.

We have described the ability of the heart to transiently switch substrate utilization and alter metabolic gene expression in response to a stimulus, which we refer to as metabolic flexibility (29). In this study, we also demonstrate a similar fluctuation in the transcriptional profile of nonmetabolic genes of the right ventricle in response to hypoxia. Our findings suggest that gene expression of the right ventricle is a dynamic process that can shift between adult and fetal iso-gene expression rapidly. In our model, early hypobaric hypoxia downregulates PPARα, MCAD, and SERCA2a transcript levels, consistent with a fetal transcriptional profile. At day 14, there is restoration of adult iso-gene expression (except for ANF transcript levels). In fact, the GLUT isoform expression pattern at 2 wk is an accentuation of the adult transcriptional profile. Our results indicate that the right ventricle demonstrates “transcriptional flexibility” in response to in vivo hypobaric hypoxia. We speculate that, like the loss of metabolic flexibility in pathological states of the heart (e.g., heart failure), this loss of transcriptional flexibility is maladaptive and contributes to cardiac dysfunction.

Our results reinforce the importance of measuring gene expression patterns over time to differentiate between adaptive and maladaptive transcriptional patterns. It is easy to see that if we only measured the gene expression of the right ventricle at day 7, we could have drawn the conclusion that hypobaric hypoxia induces a fetal transcriptional profile. It is possible that even longer durations of hypoxia may eventually result in a maladaptive transcriptional profile characterized by a sustained substrate switch to glucose oxidation and persistent fetal gene expression. However, we believe that this transcriptional flexibility of the right ventricle in response to hypoxia suggests an adaptive process.

Limitations. Because we were unable to measure both RV and LV function in rats exposed to hypobaric hypoxia, we relied primarily on morphology and gene expression to determine that hypoxia-induced RV hypertrophy is adaptive. However, preliminary data in Langendorff-perfused rat hearts exposed hypobaric hypoxia suggest that there is no significant change in RV function after 1 or 2 wk of hypoxia exposure (K. Ngumbela and M. Faadiel Essop, unpublished observations). Furthermore, previous studies (18) employing a similar model of hypobaric hypoxia also failed to demonstrate a change in RV or LV function. Because of limited tissue availability, we were unable to measure protein expression in the right ventricle. However, we (30, 31) have previously demonstrated that changes in metabolic gene expression correlate very well with metabolic fluxes in the rat heart.

In conclusion, there are dynamic changes in the transcriptional response of hypoxia-induced RV hypertrophy characterized by transient reactivation of the fetal gene program. We speculate that this transcriptional flexibility is an adaptive process. We also determined that ANF, PPARα, GLUT isoforms, and SERCA2a were regulated in part by increased load, whereas PDK-4 and myosin iso-gene expression were regulated by hypoxia and/or neuroendocrine factors.

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