Myocardial aerobic metabolism is impaired in a cell culture model of cyanotic heart disease

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Myocardial aerobic metabolism is impaired in a cell culture model of cyanotic heart disease. Am. J. Physiol. 275 (Heart Circ. Physiol. 44): H1673–H1681, 1998.—A human pediatric cardiomyocyte cell culture model of chronic cyanosis was used to assess the effects of low oxygen tension on mitochondrial enzyme activity to address the postoperative increase in lactate and decreased ATP in the myocardium and the high incidence of low-output failure with restoration of normal oxygen tension, after technically successful corrective cardiac surgery. Chronically hypoxic cells (PO2 = 40 mmHg for 7 days) exhibited significantly reduced activities for pyruvate dehydrogenase, cytochrome-c oxidase, succinate cytochrome c reductase, succinate dehydrogenase, and citrate synthase. The activity of NADH-cytochrome c reductase was unaffected. Lactate production and the lactate-to-pyruvate ratio were significantly greater in hypoxic cardiomyocytes. Western and Northern analysis demonstrated a decrease in the levels of various mRNA and corresponding polypeptides in hypoxic cells. Thus hypoxia influences mitochondrial metabolism through acute and chronic adaptive mechanisms, reflecting allosteric (post-transcriptional) and transcriptional modulation. Transcriptional downregulation of key mitochondrial enzyme systems can explain the insufficient myocardial aerobic metabolism and low-output failure in children with cyanotic heart disease after cardiac surgery.

hypoxia; cardiomyocytes; mitochondria; metabolism; cytochrome-c oxidase; pyruvate dehydrogenase

Cyanotic Tetralogy of Fallot (TOF) children greater than 3 mo of age are at risk for postoperative myocardial contractile failure and have impaired metabolism resulting in decreased postoperative myocardial ATP concentrations and elevated lactate levels (9). The metabolic abnormality during reperfusion suggests that mitochondrial dysfunction may contribute to complications such as low-output syndrome and depressed ventricular function (16, 41, 52). These effects are exacerbated during the postnatal period when metabolic changes occur to the myocardium, making it more reliant on oxygen than during the fetal period (25, 26). It has been demonstrated that mitochondrial oxidative phosphorylation responds to physiological alterations in oxygen tension by altering the rate of cellular respiration (4, 6, 25, 49). The major mitochondrial enzyme system that responds to changes in oxygen tension is cytochrome-c oxidase (COX; complex IV). COX, the terminal enzyme of the respiratory chain, is reversibly inhibited by acute (<24 h) hypoxia (4, 5, 39). The effect of chronic hypoxia on COX and the other components of the respiratory chain (NADH dehydrogenase (complex I), succinate dehydrogenase (complex II), and the ubiquinone cytochrome c reductase (complex III)) has not been directly investigated, particularly in myocardial cells. It has been reported that in hepatocytes, low oxygen tension (7 mmHg) acts to reduce the assembly of various subunits that comprise complexes III and IV, which was independent of their synthesis (1). This effect occurs over a period of 24 h and is reversible. Hypoxia has also been shown to downregulate the steady-state levels of various mitochondrially encoded transcripts (14). As an example, the level of COX subunit III mRNA decreased, whereas the level of the 12S and 16S rRNA remained unchanged after 48 h at an oxygen tension of 15 mmHg (14). These data suggest that transcriptional and posttranscriptional regulatory mechanisms likely govern the synthesis, rate of assembly, and activity of mitochondrial respiratory chain components. The effect of chronically low oxygen tension (40 mmHg for 7 days) on the mitochondrial respiratory chain, the pyruvate dehydrogenase (PDH) complex, and citrate synthase (CS) in cultured human pediatric cardiomyocytes remains to be thoroughly studied and is the focus of this investigation.

MATERIALS AND METHODS

Culture of Human Cardiomyocytes

Human cardiomyocytes, cultured from tissue biopsy specimens (20 mg) obtained from pediatric patients (6 mo to 2 yr of age) undergoing corrective surgery for TOF, have previously been extensively characterized (17, 20). Briefly, the heart tissue was washed in PBS (137 mM NaCl, 2.7 mM KCl, 8.1 mM Na2HPO4, and 1.5 mM KH2PO4), and the cells were released by digestion with 0.2% trypsin-0.1% collagenase. The dissociated cells were removed with a Pasteur pipette and pelleted by centrifugation. The isolated cells were cultured in Iscove’s modified Dulbecco’s medium (GIBCO Laboratories) containing 0.1 mM β-mercaptoethanol, 10% fetal bovine serum, 10 U/ml penicillin, and 100 µg/ml streptomycin. Cells were seeded at low density, and after attachment, cardiomyocyte colonies were transfected with new culture dishes with a pipette and further propagated. Cultures exhibiting >95% purity, as assessed by fluorescent monoclonal antibody staining for human ventricular myosin heavy chain, were used for subsequent studies. Equal numbers of cells, established at a PO2 of 150 mmHg, were seeded on each plate before segregation to either a high or low oxygen tension. All procedures have been previously approved by The Toronto Hospital Ethics Committee.

In Vitro Modeling of the Cyanotic Myocardium

Chronic hypoxia was mimicked by exposing cardiomyocytes to a low oxygen atmosphere (PO2 = 40 mmHg) for a...
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An oxygen tension of ~40 mmHg was maintained within the cell culture incubator by continuous infusion with a mixture of 95% N2-5% CO2. An oxygen tension of 40 mmHg was chosen because it represents the approximate lower range of arterial oxygen tension observed in children with TOF. Normoxia in this study was defined as a constant oxygen tension of 150 mmHg. Practical considerations dictated the use of the two indicated oxygen tensions. Regulation of air and CO2 flows to obtain an oxygen tension of 40 mmHg was maintained within the cell culture incubator when equilibrated with a humidified CO2 concentration of 5%. To simulate the postoperative restoration of arterial oxygen tension, chronically hypoxic cardiomyocytes cultured at a PO2 of 40 mmHg were transferred to an oxygen tension of 150 mmHg for varying periods of time, and the desired parameters were assessed. The culture medium in normoxic and hypoxic cells was replaced twice a week. The medium used for hypoxic cells was equilibrated to an oxygen tension of 40 mmHg before use. The oxygen tension of the cell culture medium was routinely monitored using a blood-gas machine (Instrumentation Laboratories, Milan, Italy).

Enzyme Measurements

PDH. Native cellular PDH complex activity was assayed as described by Sheu et al. (40).

CS. CS activity was determined from whole cell extracts as described by Robinson et al. (37) and Merante et al. (28).

Respiratory chain enzymes. COX, complex II + III, and complex I + III activities were determined on whole cell extracts as described by Glerum et al. (12), Merante et al. (26), and Pitkänen et al. (30). All enzyme measurements were performed on a Cobas Fara analyzer (Hoffman LaRoche). Succinate dehydrogenase (SDH) activity was determined on whole cell extracts, in the presence or absence of antimycin A, using a modified protocol of Robinson et al. (37). Briefly, cells were sonicated in assay buffer (250 mM sucrose, 50 mM Tris-HCl, 1 mM EDTA, and 2.5 mM potassium cyanide, pH 7.4). To two cuvettes we added the following: 910 µl buffer, 50 µl cell extract, 10 µl of 10 mM EDTA, 16 µl of 4.65 mM dichloroindophenol (DCIP), 10 µl of 5 mM ubiquinone-2, and 1 µl of 4% Triton X-100. Antimycin A (1 µl of a 10 mg/ml stock) was added to one of the cuvettes to determine the SDH specific rate. The reaction was initiated by the addition of 20 µl of 1 M succinate. The rate of DCIP reduction was followed using a microplate reader (Titertek, Marianna, Florida). The data are represented as means ± SD.

Western Blot Analysis

Mitochondria were isolated from cells (20 plates containing 1 x 10^6 cells/plate) that had been stabilized for 7 days under either normoxic or hypoxic conditions. Mitochondria were prepared according to Pitkänen et al. (30) and resuspended in 100 µl isolation buffer (0.25 M sucrose, 20 mM Tris, and 1 mM EDTA, pH 7.0). An aliquot was removed and used to determine protein concentration. The remaining mitochondria were solubilized in 2x gel loading buffer (125 mM Tris-HCl, pH 6.5, 4% SDS, 20% glycerol, and 573 µM β-mercaptoethanol) and used for Western analysis (27). Fifty micrograms of each protein sample were fractionated through a 4% stacking and 10% running gel and transferred onto a polyvinylidifluoride membrane according to Ausubel et al. (2). The membranes were blocked in 1x TTBS (10 mM Tris-HCl, pH 7.5, 100 mM NaCl, and 0.1% Tween 20) containing 5% nonfat dried milk for 1 h and subsequently reacted with the desired antibody for 1 h in the same buffer. The antibodies used in this study were as follows: mouse anti-COX subunit IV (1 µg/ml; Molecular Probes; Tannman et al., Ref. 43), rabbit anti-Holo-COX polyclonal (used at a 1500 dilution), and rabbit anti-Elx PDH polyclonal (1500 dilution). Polyclonal antibodies used in this study were generated in the laboratory of Dr. B. H. Robinson (Hospital for Sick Children, Toronto, Ontario, Canada). The blots were washed twice for 15 min each in 1x TTBS and reacted against goat anti-rabbit or anti-mouse secondary antibodies conjugated to alkaline phosphatase (Bio-Rad) for colorimetric detection.

Statistical Analysis

All data were analyzed by the Student's t-test for two group comparisons (unpaired analysis) or by ANOVA where applicable. The data are represented as means ± SD.

RESULTS

Modulation of the Cellular Redox State

Cardiomyocytes maintained under chronically hypoxic conditions (7 days) released more lactate (727.52 ± 80.43 vs. 442.00 ± 82.88 nmol·min⁻¹·mg⁻¹) at 40 and 150 mmHg, respectively; P < 0.006 by t-test; Fig. 1A) into the incubation medium and released significantly less pyruvate (4.11 ± 0.97 vs. 13.45 ± 2.75 nmol·min⁻¹·mg⁻¹, P < 0.0008; Fig. 1B), relative to normoxic cells, when provided with 1 mM glucose for 1 h. This resulted in L/P values of 189.09 ± 68.28 vs. 33.92 ± 8.14 for oxygen tensions of 40 and 150 mmHg, respectively (Fig. 1C).
PDH, CS, SDH, and Respiratory Chain Enzyme Activities

To investigate the effects of chronic hypoxia on mitochondrial metabolism, the activities of PDH, CS, COX, complexes I + III, II + III, and SDH were investigated (Figs. 2 and 3). The levels of PDH (0.17 ± 0.08 vs. 0.61 ± 0.09 nmol·min⁻¹·mg⁻¹ at 40 and 150 mmHg, respectively; P < 0.001 by ANOVA; Fig. 2A) and COX (6.99 ± 1.67 vs. 34.90 ± 7.74 nmol·min⁻¹·mg⁻¹; P < 0.0001; Fig. 3A) were significantly decreased in hypoxic cells. The level of complex II + III activity was also reduced (9.71 ± 3.02 vs. 18.25 ± 2.08 nmol·min⁻¹·mg⁻¹; P < 0.001; Fig. 3B), as was the level of SDH (8.13 ± 2.90 vs. 12.57 ± 3.35 µmol·min⁻¹·mg⁻¹; P < 0.05; Fig. 3C) and CS (59.80 ± 7.59 and 99.29 ± 8.22 nmol·min⁻¹·mg⁻¹; P < 0.05; Fig. 2B). The activity of complex I + III (143.60 ± 29 vs. 145.48 ± 36.40 nmol·min⁻¹·mg⁻¹; Fig. 3D) was not influenced by oxygen tension.

Oxygen Modulation of COX Activity During Culture

Changes to COX activity during culture were assessed under normoxic and hypoxic conditions. COX activity was assayed at 1, 3, 5, and 7 days. Under normoxic conditions, COX activity progressively increased from 6.62 ± 2.07 nmol·min⁻¹·mg⁻¹ and plateaued at day 5 (31.47 ± 1.16 nmol·min⁻¹·mg⁻¹). The hypoxic cardiomyocytes exhibited significantly reduced COX activity over the same period (Fig. 4).

Kinetics of PDH and Respiratory Chain Modulation by Changing Oxygen Tension

Figure 5 shows the change in enzyme activities over a 24-h period following an alteration in oxygen tension. When cells were transferred from a high to a low oxygen tension, PDH activity declined within 6 h (0.53 ± 0.04 at 0 h vs. 0.31 ± 0.05 and 0.34 ± 0.08 nmol·min⁻¹·mg⁻¹ at 6 and 24 h, respectively; P < 0.001, 0 vs. 6 and 24 h). In contrast, no significant change to PDH activity was observed over a 24-h period when the converse experiment was performed, whereby cells from a hypoxic atmosphere were transferred to normoxia (0.20 ± 0.04 at 0 h and 0.18 ± 0.04 and 0.23 ± 0.06 nmol·min⁻¹·mg⁻¹ at 6 and 24 h, respectively). Transferring cells from a normoxic to a hypoxic atmosphere resulted in a significant decline in COX activity (35.84 ± 6.86 at 0 h to...
19.98 ± 3.98 at 24 h; P < 0.0001, 24 vs. 0 and 6 h). A slight but nonsignificant decrease in complex II + III activity (17.10 ± 3.77 at 0 h to 15.40 ± 1.97 at 24 h) was also noted after 24 h. Conversely, a significant increase in COX (7.75 ± 1.80 at 0 h to 11.97 ± 2.56 at 24 h; P < 0.0001, 24 vs. 0 and 6 h) and complex II + III (8.03 ± 1.85 at 0 h to 9.43 ± 2.53 at 6 h and to 15.16 ± 3.00 at 24 h; P < 0.0001, 6 vs. 24 h and P < 0.0001, 24 vs. 0 and 6 h) activity was observed when cells were transferred from a hypoxic to a normoxic oxygen atmosphere. No significant change occurred to complex I + III activity over 6 or 24 h as a function of oxygen tension (Fig. 5D).

Western Blot Analysis of PDH and COX Subunits

Western blot analysis performed on isolated mitochondria was used to determine the steady-state levels of COX and PDH subunits. Figure 6 shows that the steady-state level of the PDH-E1α subunit, COX subunit I, and COX subunit IV were reduced in mitochondria isolated from cells maintained at a low oxygen tension. A similar decrease in the steady-state abundance of PDH subunits was observed when Western analysis was performed using total cell extracts (Fig. 6B).

Northern Blot Analysis

The steady-state level of COX I, PDH-E1α, GAPDH, and the 18S rRNA transcripts were assessed under conditions of normoxia and hypoxia. The transcript levels for COX I and PDH-E1α were reduced in hypoxic cells relative to normoxic cells (Fig. 7, A and B). The level of the 18S rRNA transcript was not altered by exposure to hypoxia, nor was the level of GAPDH (Fig. 7C).

DISCUSSION

Cyanotic children produce more lactate during the course of cardiac surgery, and these levels remain elevated 30 min postreperfusion, compared with noncyanotic patients (9). Myocardial ATP concentration is also significantly reduced in cyanotic patients after corrective surgery compared with normoxic patients (9). These metabolic abnormalities may explain the increased risk of contractile dysfunction necessitating
ionotropic support in the patient postoperatively. Excessive lactate production may likely reflect myocardial adaptation to chronic cyanosis (31, 41, 43) and has been noted in animal and cell culture models of hypoxia (1, 13, 14, 25, 31, 39, 48, 51). In part, elevated lactates reflect altered mitochondrial metabolism (13, 50), which would contribute to the depletion of cellular ATP and an inability to replenish ATP stores postoperatively (31, 41, 44). In a clinical setting, the reduced capacity for aerobically derived ATP would limit myocardial recovery after surgery. It has been shown that the recovery of myocardial ATP pools requires considerable time for recovery after an ischemic insult (47). Because hypoxic children exhibit significantly decreased levels of ATP after surgery (9), the ability to replenish myocardial ATP stores is compromised, possibly leading to a greater incidence of postsurgical complications.

To investigate the effects of cyanosis on human cardiomyocytes, a cell culture model of chronic hypoxia was used. The advantage of this system includes the homogeneity of cells (17, 20) and the ease by which interventions could be assessed. Primary human cardiomyocytes in culture allow for an adequate stabilization period before measuring the desired parameters. These nonbeating cultures mimic cardiopically arrested heart cells at the time of surgery. A disadvantage of

Fig. 4. Time course for COX activity after subculture. Cardiomyocyte cultures were subdivided and placed at either a normoxic (open bars) or a hypoxic (solid bars) oxygen environment. COX activity was determined at days 1, 3, 5, and 7 after subculture. COX activity is expressed as nmol·min⁻¹·mg total cellular protein⁻¹. Data are means ± SD; n = 3 plates/group. @P < 0.05, **P < 0.0001.

Fig. 5. Short-term time course (<24 h) of PDH and respiratory chain enzyme activities after reciprocal exchange. Cardiomyocyte cultures stabilized at 150 mmHg for 7 days were transferred to 40 mmHg, and enzyme activities were determined at 0, 6, and 24 h. The converse was also performed whereby cultures stabilized at an oxygen tension of 40 mmHg were transferred to 150 mmHg and enzyme activities were determined as outlined. Values are represented as nmol·min⁻¹·mg total cellular protein⁻¹. Data are means ± SD; n = 6 plates/group. A: PDH (*P < 0.01, 40 vs. 150 mmHg). B: COX (**P < 0.0001, 24 vs. 0 and 6 h). C: complex II + III (#P < 0.01, 24 vs. 0 and 6 h). D: complex I + III (no significant change).
E1α (loading consistency. stripped and rehybridized to a human 18S rDNA probe to assess hybridized to 32P-labeled probes corresponding to COX I, COX IV, and GAPDH transcripts. Total cellu-
ral protein or total cellular protein were isolated from hypoxic (40 mmHg) and normoxic (150 mmHg) cells. Proteins were fractionated through a 4% stacking and 10% running gel and transferred onto a polyvinylidifluoride membrane. Blots were individually reacted with antibodies specific for PDH-E1α, holoCOX, or COX subunit IV.

using nonbeating cardiomyocytes from children is that functional contractility assessments cannot be performed and the metabolic effects on function during reperfusion cannot be readily determined. This limitation is superseded by the availability of this human neonatal cardiac tissue, which is routinely excised during the surgical repair of TOF. The energy require-
tion is superseded by the availability of this human neonatal cardiac tissue, which is routinely excised during the surgical repair of TOF. The energy require-
ments for beating cells would be greater than those for the quiescent cells used in this investigation. The cultures maintained at a PO2 of 40 mmHg did not show any overt phenotypic change in size or any loss of viability as a result of culturing at a low oxygen tension (17, 20).

Metabolic changes that occur to mitochondria during hypoxia (>48 h) involve both transcriptional and post-
transcriptional effects. We have demonstrated by North-
ern analysis that COX I, a mitochondrially encoded transcript and PDH-E1α, a nuclear encoded transcript, are downregulated under conditions of chronic hypoxia. In contrast, the GAPDH transcript, a nuclear-encoded cytoplasmically resident glycolytic enzyme, is unaffected by hypoxia. In accordance with the decline in mRNA levels for both COX and PDH transcripts, a corresponding decrease was seen for their encoded polypeptides as determined by Western blot analysis.

For example, the level of COX subunit IV (a nuclear-encoded subunit), COX I, and E1α polypeptides are decreased in hypoxic cells. The observed decrease in the steady-state level of subunits, as assessed in both whole cell and in mitochondrial extracts, suggests that physically less COX and PDH complexes exist within the mitochondria as an adaptation response to hypoxia.

A further level of posttranslational regulation may fine-tune the cells’ adaptation to hypoxia. Complex III activity, for example, has been shown to be downregu-
lated posttranscriptionally by mechanisms that involve the rate of enzyme assembly and allosteric modulation (1, 14). Purified COX responds to changes in oxygen tension that are well above the level required for enzyme saturation (3, 4, 50) and are above the critical value of 1–10 mmHg as determined for isolated rat hepatocytes (39). This reversible, allosteric inhibition of COX activity occurs progressively within a 1-h period at 20 mmHg. We have shown that a significant decrease in cellular COX activity occurs at 40 mmHg in cardio-
myocytes, which is in the physiological arterial oxygen range of patients with cyanotic heart disease.

It is well established that functional changes occur to mitochondria during neonatal development. During this stage, mitochondrial functions change from a fetal to a newborn state (6). This maturation coincides with an increase in the level of cytochromes (24), with COX developmental isoform switching (10, 21, 38) and a greater dependence on aerobically produced ATP after the first week of life (22, 23). Thus, in the chronically hypoxic newborn, the high dependence for glycolysis may still be operative as a means to compensate for reduced mitochondrial capacity. The chronically hypoxic cardiomyocytes used in this study produce more lactate than control cells, possibly reflecting a greater need for glucose oxidation to generate ATP. This is also reflected in the dramatically altered L/P, indicating that the cells are functioning at an altered redox state. Elevated L/P values are indicative of an impairment to PDH or respiratory chain activity (33, 35, 36). This study determined that both PDH and COX activities are decreased in hypoxic cardiomyocytes, thereby accounting for the altered cellular redox state. The alteration of the cellular L/P correlates with changes to the NAD+/NADH (34). Schumacker et al. (39) have shown that NAD(P)H levels increase during hypoxia with a concomitant decrease in the respiratory rate. The correspondingly low PDH activity may be further augmented by the reduced flux through COX. For example, the allosteric modulation of PDH may be mediated, in part, by the increased NAD(P)H levels present in hypoxic cells (32) that occur as a consequence of COX impairment (34). It has been shown that increased levels of intracellular NADH act to inhibit the PDH complex (29). The chronically hypoxic myocardium perturbs the efficiency of lactate extraction, resulting in a concomitant increase in the L/P (11). The regulatory influence of hypoxia on the PDH complex activity, both transcriptionally and allosterically, has not been previously reported to our knowledge.
The decreased level of complex II + III activity, but normal complex I + III activity, suggested that complex III activity was not rate limiting and that SDH (complex II) was influenced by oxygen tension. This observation was verified by directly measuring SDH. The novel observation that complex I + III activity is maintained under low oxygen tension in cardiomyocytes is at odds with the observation that complex I is inhibited by 30% in hypoxic mouse cerebral cortex (6). This variation may reflect tissue-specific differences (i.e., isoform composition) and regulation of complex I between heart and brain.

The period of time required for COX activity to stabilize after subculture mirrors what we had previously reported for glutathione peroxidase (7). The level of COX activity under normoxic conditions at day 1 after subculture was low. The activity progressively increased to the expected control levels by day 5 of culture. In contrast, the level of COX activity under hypoxic conditions remains depressed throughout the culture period.

The kinetics at which the COX and PDH activities decrease immediately after a shift to a low oxygen atmosphere most likely reflects posttranslational and allosteric regulation of the respective complexes. For example, the normoxic PDH activity was significantly decreased within 6 h after a hypoxic shift. This rapid response may be mediated by the activation of the PDH kinase that acts to phosphorylate the E1α subunit and inactivate the complex (29, 34). Alternatively, the inhibition of the PDH phosphatase would yield a similar result. Interestingly, the time required to see PDH inhibition was more rapid than the time required to see a corresponding inhibition of COX activity. Thus a mechanism must exist that mediates PDH repression in response to changing oxygen tension, independent of the respiratory chain. An allosteric mechanism mediating the early phase of PDH inhibition is favored since the PDH complex can respond quickly to other insults such as ischemia-reperfusion (15). In this event, the complex is dramatically inhibited within 2 min after the onset of reperfusion. Incidentally, the inhibition of PDH activity at low oxygen tension was not counteracted by the chronic (24 h) or acute (15 min) administration of dichloroacetate (DCA) to the cells (data not shown). DCA activates the PDH complex by inhibiting the PDH kinase and preventing phosphorylation-induced allosteric inhibition (42). DCA was able to augment the basal level of cardiomyocyte PDH activity by ~30% in both normoxic and hypoxic cells (data not shown). Thus chronically hypoxic cardiomyocytes maintain a reserve of PDH activity that may represent an equilibrium between the control exerted by the kinase and phosphatase regulatory subunits.

Allosteric regulation is also implicated for the change in complex II + III activity. When cardiomyocytes were shifted from normoxia to hypoxia, a slight but nonsignificant decrease in activity occurred over 24 h. In the converse experiment, when cells were transferred from a hypoxic to normoxic atmosphere, near-control activities were established within 24 h. In accordance with the original long-term (7-day) observations for complex I + III activity, reciprocal exchange experiments did not show any variation as a function of hypoxia. Thus, in contrast to observations in the brain, we observe that complex I in cardiomyocytes is relatively unresponsive to physiological changes to oxygen tension.

From these observations, it appears that two main control points mediating mitochondrial adaptation to chronic hypoxia occur at the PDH and COX complexes. This is not surprising because PDH is the primary gateway for the entry of tricarboxylic acid cycle intermediates into the mitochondria and COX mediates the terminal, rate-limiting step, in oxidative phosphorylation.

We have shown that key mitochondrial enzymes, PDH and COX, are downregulated by chronic hypoxia in cardiomyocytes. This repression occurs in two phases: an acute phase that responds to low oxygen tension over a course of hours to allosterically inhibit mitochondrial enzyme activity and a long-term adaptation that acts to alter the steady-state level of mitochondrial enzymes. The role of transcriptional modulation has been verified by Northern and Western analysis, whereby a reduction in mRNA and polypeptides is evident. The factor(s) sensing and mediating these sets of events remains to be characterized but may be mediated in part by hypoxia-responsive transcription factors (46) and oxygen-responsive promoter elements (8).

Thus the transcriptional down-regulation of key aerobic mitochondrial enzymes is the likely mechanism by which postoperative myocardial complications of low-output syndrome occur in cyanotic children undergoing cardiac surgery. By understanding the events mediating these responses to chronic hypoxia, interventions can be devised to augment mitochondrial function in a clinical setting. The molecular mechanisms by which these events occur are currently under active investigation. This knowledge may allow for active metabolic intervention to improve the survival and decrease the morbidity of hypoxic children undergoing cardiac surgery.

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