Persistent pulmonary hypertension results in reduced tetralinoleoyl-cardiolipin and mitochondrial complex II + III during the development of right ventricular hypertrophy in the neonatal pig heart

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Persistent pulmonary hypertension results in reduced tetralinoleoyl-cardiolipin and mitochondrial complex II + III during the development of right ventricular hypertrophy in the neonatal pig heart. Am J Physiol Heart Circ Physiol 301: H1415–H1424, 2011. First published August 12, 2011; doi:10.1152/ajpheart.00247.2011.—Persistent pulmonary hypertension of the newborn (PPHN) results in right ventricular (RV) hypertrophy followed by right heart failure and an associated mitochondrial dysfunction. The phospholipid cardiolipin plays a key role in maintaining mitochondrial respiratory and cardiac function via modulation of the activities of enzymes involved in oxidative phosphorylation. In this study, changes in cardiolipin and cardiolipin metabolism were investigated during the development of right heart failure. Newborn piglets (≤24 h old) were exposed to a hypoxic (10% O2) environment for 3 days, resulting in the induction of PPHN. Two sets of control piglets were used: 1) newborn or 2) exposed to a normoxic (21% O2) environment for 3 days. Cardiolipin biosynthetic and remodeling enzymes, mitochondrial complex II + III activity, incorporation of [1-14C]linoleoyl-CoA into cardiolipin precursors, and the tetralinoleoyl-cardiolipin pool size were determined in both the RV and left ventricle (LV). PPHN resulted in an increased heart-to-body weight ratio, RV-to-LV plus septum weight ratio, and expression of brain naturetic peptide in RV. In addition, PPHN reduced cardiolipin biosynthesis and remodeling in the RV and LV, which resulted in decreased tetralinoleoyl-cardiolipin levels and reduced complex II + III activity and protein levels of mitochondrial complexes II, III, and IV in the RV. This is the first study to examine the pattern of cardiolipin metabolism during the early development of both the RV and LV of the newborn piglet and to demonstrate that PPHN-induced alterations in cardiolipin biosynthetic and remodeling enzymes contribute to reduced tetralinoleoyl-cardiolipin and mitochondrial respiratory chain function during the development of RV hypertrophy. These defects in cardiolipin may play an important role in the rapid development of RV dysfunction and right heart failure in PPHN.

Heart failure; mitochondria; cardiomyopathy; lipid; metabolism

Persistent pulmonary hypertension of the newborn (PPHN) is a clinical symptom associated with 10–30% of mortality and up to 6 per 1,000 incidence of live births (55, 56). It is characterized by high pulmonary vascular resistance due to the failure of normal pulmonary vascular relaxation at or shortly after birth (7, 28, 37). Different underlying mechanisms, including perinatal hypoxia, inflammation, direct lung injury, and meconium aspiration, have been described as the major causes of PPHN development. Although primary changes are observed in the lungs, secondary disturbances in cardiac performance may perturb the fine equilibrium between the systemic and pulmonary circulation in PPHN (27). The elevated pulmonary resistance in PPHN increases right ventricular (RV) afterload and impairs O2 delivery, leading to RV dysfunction (11). The development of RV hypertrophy followed by heart failure (HF) is considered as the main limiting factor for the survival of infants with PPHN (11, 27).

Cardiolipin (CL) is a major phospholipid of the mitochondrial inner membrane. The appropriate content and fatty acyl composition of CL are required for optimal activity of a number of key enzymes involved in oxidative phosphorylation (4, 14, 47). CL is essential for mitochondrial biogenesis and the assembly of respiratory enzyme supercomplexes (5, 39). Cardiac CL is synthesized in a series of steps from phosphatidic acid (PA) and is then remodeled by a two-step deacylation-reacylation process or by transacylation into a form that is linoleic acid enriched (1, 13, 33, 53, 54, 57). Tetralinoleoyl-CL (L4-CL) comprises the majority of CL in the human and mammalian heart (46, 49). We (43) recently observed significant reductions in L4-CL and alterations in CL biosynthetic and remodeling processes in left ventricular (LV) hypertrophy and subsequent HF in spontaneous hypertensive HF (SHHF) rats and in LV human heart explants isolated from HF patients. Because of a previous study (6) that indicated that impairment of mitochondrial energy-producing ability was involved in the development of RV failure in monocrotaline-induced pulmonary hypertensive rats, we wanted to examine whether alterations in L4-CL and CL biosynthetic and remodeling processes occurred in the RV during the development of cardiac hypertrophy and HF. Indeed, our results show that PPHN results in RV alterations in CL biosynthetic and remodeling enzymes that lead to a decrease in L4-CL, resulting in reduced mitochondrial complex II + III activity, establishing CL as a potentially important link between PPHN and the development of RV failure.
MATERIALS AND METHODS

[14C]glycerol-3-phosphate, 1-acyl, 2-[1-14C]arachidonyl-glycerophosphorylcholine, phosphatidyl[methyl-3H]choline, [5-3H]CTP, and [1-14C]-linoleoyl-CoA were obtained from either Dupont (Mississauga, ON, Canada) or Amersham (Oakville, ON, Canada). Phosphatidyl[3H]glycerol was synthesized from [14C]glycerol-3-phosphate as previously described (17). Lipid standards were obtained from Serdery Research Laboratories (Englewood Cliffs, NJ). Thin-layer chromatographic plates (silica gel G, 0.25 mm thickness) were obtained from Fisher Scientific (Winnipeg, MB, Canada). Ecolite scintillant was obtained from ICN Biochemicals (Montreal, QC, Canada). Mitoprofile total oxphos rodent Western blot antibody cocktail (MS 604) was obtained from Mitosciences (Eugene, OR). Secondary monoclonal anti-mouse antibodies were obtained from Perkin-Elmer (Woodbridge, ON, Canada). The Western blot analysis system was used for protein expression experiments and was obtained from Amersham Pharmacia Biotech UK (Buckinghamshire, UK). Kodak X-OMAT film was obtained from Eastman Kodak (Rochester, NY). All other chemicals were certified American Chemical Society grade and obtained either from Fisher Scientific or Sigma Chemical (St. Louis, MO).

PPHN model. Experiments were in accordance with the guidelines of the Canadian Council on Animal Care and were approved by the animal protocol committee of the University of Manitoba. Newborn piglets (<24 h old, n = 4) were killed on the day of arrival from the farm supplier. Newborn piglets (<24 h old, n = 4) were exposed to 21% (control) or 10% (PPHN) O2 for 3 days after birth in a normobaric chamber as previously described (8, 23, 24). Animals were fed ad libitum with an artificial sow milk replacer obtained commercially from Feedrite (Winnipeg, MB, Canada). Animals were euthanized by a pentobarbital overdose and exsanguination, and the heart was removed and placed in oxygenated, cold (4°C) Ca2+-free Krebs-Henseleit physiological buffer [containing (in mM) 112.6 NaCl, 25 NaHCO3, 1.38 NaH2PO4, 4.7 KCl, 2.46 MgSO4,7H2O, and 5.56 dextrose; pH 7.4]. RV afterloading was determined by the

Table 1. Sequence of the porcine primers used for real-time PCR

<table>
<thead>
<tr>
<th>Target Gene</th>
<th>Forward Primer</th>
<th>Reverse Primer</th>
<th>Product Length, bp</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tafazzin</td>
<td>5'-TGCCTCTCTGCGCTTTCGGCTTCC-3'</td>
<td>5'-ACTGCTTCTGCGCTACTTCC-3'</td>
<td>107</td>
</tr>
<tr>
<td>Brain natriuretic peptide</td>
<td>5'-GTCTGCTGCTGCTGCTTGCTTCC-3'</td>
<td>5'-GTCAGTCGCTGCTGCTTCC-3'</td>
<td>108</td>
</tr>
<tr>
<td>Ca2+-independent phospholipase A2γ</td>
<td>5'-AGACCGAAACACGACCACTG-3'</td>
<td>5'-CATCTGGACACCGACCACTG-3'</td>
<td>115</td>
</tr>
<tr>
<td>β-Actin</td>
<td>5'-GCTGCTGAGAAGCTGTTGACAG-3'</td>
<td>5'-ATCCAGAGTGTCGCAAGAAG-3'</td>
<td>104</td>
</tr>
</tbody>
</table>

SYBR green I can detect specific and nonspecific PCR products since it can bind to double-stranded DNA in a sequence-independent manner (10). Thus, a melting curve that increased in temperature incrementally from to 60 to 95°C over the course of 20 min was performed at the end of each experiment to confirm the absence of primer-dimers in specific PCR products. The primer sequences used are shown in Table 1. Changes in gene expression were analyzed with Eppendorf Mastercycler ep realplex software (version 1.5.474). Data are presented as mean fold changes (2-∆∆Ct, where Ct is threshold cycle) in mRNA expression (29) relative to β-actin (9). mRNA expression from 3-day-old control and PPHN piglets was compared relative to newborn piglets, which were assigned a value of 1 (19).

Preparation of subcellular fractions and assay of enzyme activities. Mitochondrial fractions were prepared as previously described (43). CDP-diacylglycerol synthetase (CDS), phosphatidylglycerophosphate synthase (PGPS), CL synthase (CLS), monolysocardiolipin (MLCL) acyltransferase (MLCL AT), and citrate synthase activities were determined as previously described (17, 19, 53). In other experiments, 20 μg of mitochondrial protein were assayed for [14C]PA, [14C]phosphatidylglycerol ([14C]PG), and [14C]MLCL production in the presence of 70,000 disintegrations/min per nanomole of [1-14C]linoleoyl-CoA as previously described (43). The activity of complex II + III (succinate cytochrome c reductase), measured as the reduction of cytochrome c after the addition of succinate, was determined as previously described (44).

Western blot analysis. Mitochondrial protein (7.5 μg) from newborn and 3-day-old control and PPHN piglets was separated on the Bio-Rad mini gel electrophoresis system by SDS-PAGE (10% acrylamide) as previously described (45). The electrophoresis was performed using synthetic pre-stained molecular markers from Bio-Rad. After electrophoresis, proteins were transferred onto polyvinylidene difluoride membranes using Tris-glycine buffer (pH 8.3) with 20% methanol at 15 V for 1.5 h. Proteins were probed overnight with Mitoprofile total oxphos rodent Western blot antibody cocktail (53 kDa, ATP synthase subunit α; 47 kDa, complex III subunit 2; 39 kDa, complex IV subunit 1; 30 kDa, complex II subunit 1p; and 20 kDa, complex I subunit NDUF8) and then probed with anti-mouse secondary monoclonal antibodies following the manufacturer’s instructions. Proteins were visualized on X-OMAT film by chemiluminescence using a Perkin-Elmer Western blot detection system. The relative intensities of the protein bands were analyzed by scanning the film in a densitometer and subsequently determined using AlphaEaseFC software.

Other determinations. CL content in mitochondrial fractions was estimated by analysis of phospholipid phosphorus as previously described (41). L-CL was determined by electrospray ionization mass spectrometry as previously described (49, 50). Protein content was determined as previously described (30). Data are expressed as means ± SE. Differences between two groups were evaluated by Student’s t-test. Data from more than two groups were evaluated by one-way ANOVA followed by a Newman-Keuls multiple-comparison test. P values of <0.05 were considered statistically significant.

RESULTS

Establishment of PPHN and RV hypertrophy. Piglets underwent percutaneous femoral catheterization after 72 h of expo-
sure to normoxic [fraction of inspired $O_2$ (Fi$_{O_2}$): 0.21] or hypoxic (Fi$_{O_2}$: 0.10) conditions. Pa$_{O_2}$ was measured in femoral arterial blood. A significant decrease in systemic Pa$_{O_2}$ was observed in PPHN animals compared with controls (Fig. 1A). Smooth muscle layer hypertrophy in elastic and muscular pulmonary arteries, resulting in wall thickening and luminal narrowing, was observed in PPHN animals compared with controls (Fig. 2). This degree of pulmonary arterial remodeling is diagnostic of PPHN. The development of PPHN was further confirmed by an increase in the RV-to-LV plus septum weight ratio over the first 3 days of life, predominately due to increased RV weight due to RV afterload as a result of PPHN (Fig. 1B). In contrast, normoxic piglets exhibited the developmentally appropriate decrease in the RV-to-LV plus septum weight ratio in the first 3 days of life. Histological examination of cardiac tissue revealed that PPHN piglets did not develop significant hyperplasia (increased density of nuclei) compared with normoxic controls (Fig. 3). Brain natriuretic peptide (BNP) is predominately secreted by ventricles, and, under hypoxic conditions, its release and levels in blood are directly proportional to ventricular volume expansion and pressure overload (26). BNP mRNA expression was increased in the RV, but not the LV, of PPHN animals compared with controls (Fig. 1C). BNP mRNA expression in the RV and LV of PPHN animals was decreased compared with newborn piglets, indicating an appropriate developmental change. Thus, hypoxia-treated piglets exhibited PPHN and the resulting RV hypertrophy.

*CL de novo biosynthesis is decreased in PPHN.* To investigate alterations in CL biosynthesis under PPHN and normal development, the activities of the three main enzymes involved in CL biosynthesis (CDS, PGPS, and CLS) were examined in control and PPHN piglets. To determine if there were regional differences in the heart, the above enzymes were examined in the RV and LV. In addition, the production of CL phospholipid precursors from [1-14C]linoleoyl-CoA were determined in mitochondrial fractions prepared from the LV and RV.

CDS activity was unaltered in the LV but decreased 56% in the RV of 3-day-old control piglets compared with newborn animals (Fig. 4A). CDS enzyme activity was decreased 34% in the RV and 31% in the LV of PPHN animals compared with controls. Incorporation of [1-14C]linoleoyl-CoA into [14C]PA in mitochondrial fractions was unaltered in the LV and RV of PPHN animals compared with controls (Table 2). Thus, regional differences in CDS activity occur during development, and CDS activity is reduced in PPHN animals. In addition, [14C]PA synthesis from [1-14C]linoleoyl-CoA appears to be normal in the mitochondria of PPHN animals.

PGPS activity was unaltered in the LV but increased by greater than twofold in the RV in 3-day-old control animals compared with newborn piglets (Fig. 4B). PGPS enzyme activity was decreased 34% in the LV and 50% in the RV of PPHN piglets compared with control animals. [1-14C]linoleoyl-CoA incorporated into PG was increased 40% in the LV and 82% in the RV of 3-day-old control animals compared with newborn piglets (Table 2). [1-14C]linoleoyl-CoA incorporated into [14C]PG was decreased 21% in the RV of PPHN piglets compared with control animals. Thus, regional differences in PGPS activity occur during development and in PPHN animals. In addition, synthesis of the CL precursor PG is reduced in PPHN.

CLS activity was unaltered in the LV and decreased 27% in the RV of 3-day-old animals compared with newborn piglets (Fig. 4C). CLS activity was decreased 62% in the LV and 50% in the RV of PPHN piglets compared with normoxic control animals. These data indicate that regional differences in CLS
activity occur during development and in PPHN animals. Together, the above results seem to indicate a net reduction in the synthesis of CL in the RV and LV of PPHN animals.

Mitochondrial CL remodeling is decreased in PPHN. To determine whether changes in CL remodeling also occur during PPHN, three enzymes involved in CL remodeling were studied: MLCL AT, Ca2+-independent phospholipase A2 (iPLA2), and tafazzin (TAZ). Mitochondrial MLCL AT activity was unaltered in the LV and RV with development in 3-day-old animals compared with newborn piglets (Fig. 5A). In contrast, MLCL AT activity was decreased 56% in the LV but unaltered in the RV of PPHN piglets compared with control animals. iPLA2 mRNA expression was increased by greater than sixfold in both the LV and RV of 3-day-old animals compared with newborn piglets (Fig. 5B). In contrast, iPLA2 mRNA expression was decreased 52% in the LV and 87% in the RV of PPHN piglets compared with control piglets. TAZ mRNA expression was unaltered in the LV and increased twofold in the RV in 3-day-old animals compared with newborn piglets (Fig. 5C). TAZ mRNA expression was unaltered in the LV but decreased 46% in the RV of PPHN piglets compared with control animals. The incorporation of [1-14C]linoleoyl-CoA into MLCL was decreased 14% in the LV and 10% in the RV of PPHN animals compared with control piglets. Together, the above results indicate a net reduction in the remodeling of mitochondrial CL in both the RV and LV of PPHN animals.

L4-CL is decreased in PPHN. The pool size of CL, as estimated by lipid phosphorus analysis, was decreased 21% in the LV and 48% in the RV of PPHN piglets compared with control animals (data not shown). L4-CL was then examined by the more sensitive electrospray ionization mass spectroscopy. L4-CL content was unaltered in the LV and RV in 3-day-old animals compared with newborn piglets (Fig. 6A). In contrast, L4-CL was decreased 39% in the LV and 60% in the RV of PPHN piglets compared with control animals. Thus, a net reduction in L4-CL was observed in both the LV and RV of PPHN animals.

Complex II + III activity is decreased in PPHN. Complex II + III activity was unaltered in the LV and RV in 3-day-old animals compared with newborn piglets (Fig. 6B). In contrast, complex II + III activity was decreased 56% in the LV and 52% in the RV of PPHN piglets compared with control animals. The alteration in complex II + III activity was not due to a decrease in mitochondrial number, as citrate synthase activity was unaltered in the LV and RV of PPHN piglets compared with 3-day-old control animals (Fig. 6C). The above
results indicate a reduction in mitochondrial respiratory chain function in the RV and LV of PPHN animals.

To determine if the decrease in complex II + III activity in the LV and RV of PPHN piglets was due to an alteration in the level of mitochondrial respiratory proteins, the protein levels of ATP synthase subunit α, complex III subunit core 2, complex IV subunit 1, complex II subunit 1p, and complex I subunit NDUFB8 were examined in the LV and RV of newborn, control, and PPHN piglets by Western blot analysis. The relative protein levels of ATP synthase subunit α, complex III subunit core 2, complex IV subunit 1, complex II subunit 1p, and complex I subunit NDUFB8 in the LV and RV were unaltered between newborn and 3-day-old piglets (Fig. 7). In contrast, the relative protein levels of complex II subunit 1p, complex III subunit core 2, and complex IV subunit 1 were significantly reduced 56%, 68%, and 75% in the RV of PPHN piglets compared with controls (n = 3). Although the relative protein levels of complex I in the RV appeared reduced, this was not statistically significant. In addition, although these complexes in the LV appeared lower, this was not statistically significant. Thus, the decrease in mitochondrial complex II + III activity in the RV of PPHN piglets was due, in part, to a reduction in the protein level of these mitochondrial complexes.

DISCUSSION

The objective of this study was to examine if there were alterations in CL biosynthetic and remodeling enzymes, L4-CL, and mitochondrial complex II + III activity in the RV during the development of PPHN with an aside to study the alteration CL-related processes during the normal developmental process in the first 3 days of life. This is the first study to date to investigate the in-depth modification of CL and related enzymes in either PPHN or newborn development. We observed regional differences in CL biosynthetic enzymes between the LV and RV of developing piglets. Specifically, CDS enzyme activity was elevated and PGPS and CLS activity were reduced in the RV, but not in the LV, of 3-day-old piglets compared with newborn animals. In addition, PGPS activity was higher in the RV than in the LV of 3-day-old piglets compared with newborn animals. It is possible that the observed differences in these enzyme activities may reflect a differential rate of RV and LV growth in the normal neonatal piglet. A previous study (38) has demonstrated that the LV free wall grows approximately three times faster than the RV free wall during the first 10 days of life in neonatal pigs. Interestingly, L4-CL content and the activities of the CL remodeling enzymes were unaltered between the LV and RV of 3-day-old piglets compared with newborn animals. These data suggest
that the level of L4-CL in the developing piglet heart may be more dependent on the level and activities of CL remodeling enzymes.

We (43, 49) have previously demonstrated that cardiac L4-CL content decreases during the progression of LV HF in rats and humans. In addition, the reduction in cardiac CL was not an aging phenomenon but was related to disease development. Cardiac hypertrophy in PPHN animals was confirmed by both an increase the RV-to-LV plus septum heart weight ratio and an increase in mRNA expression of BNP in the RV. The reduction in CL biosynthesis and remodeling activities were not due to a reduction in the number of mitochondria, as citrate synthase activity was unaltered between control and PPHN animals. We (49, 50) have previously demonstrated that cardiac citrate synthase activity is unaltered during the development of HF in SHHF rats as well. In the present study, the observed alteration in CL metabolism during the development of RV hypertrophy in PPHN appeared to be different from LV hypertrophy and HF in SHHF animals.

The results of our study on L4-CL and CL biosynthetic and remodeling genes in the RV during the development of HF in PPHN are shown in Fig. 8. De novo biosynthesis of CL in mammalian heart mitochondria is initiated by the conversion of PA to CDP-1,2-diacyl-sn-glycerol (CDP-DG) catalyzed by CDS (13). Pulse-chase labeling studies (13, 16) in the isolated rat heart demonstrated that this enzyme catalyzes a rate-limiting step in CL biosynthesis. We observed decreased CDS enzyme activity in the LV and RV of PPHN animals, indicating reduced production of CL precursors, which contribute to the lowered CL content in PPHN animals. We (43) have

Table 2. Synthesis of cardiolipin precursors and metabolites in newborn, control, and PPHN animals

<table>
<thead>
<tr>
<th>Amount Formed</th>
<th>Newborn Animals</th>
<th>Control Animals</th>
<th>PPHN Animals</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>LV</td>
<td>RV</td>
<td>LV</td>
</tr>
<tr>
<td>Phosphatidic acid</td>
<td>13 ± 7</td>
<td>22 ± 7</td>
<td>8 ± 4</td>
</tr>
<tr>
<td>Phosphatidylglycerol</td>
<td>55 ± 7</td>
<td>51 ± 5</td>
<td>77 ± 6*</td>
</tr>
<tr>
<td>Monolysocardiolipid</td>
<td>229 ± 15</td>
<td>245 ± 10</td>
<td>224 ± 4</td>
</tr>
</tbody>
</table>

Values are means ± SE (in pmol·min⁻¹·mg⁻¹). LV, left ventricle; RV, right ventricle. *P < 0.05 vs. newborn animals; †P < 0.05 vs. control animals.
previously demonstrated that during the development of LV hypertrophy in SHHF rats, CDS enzyme activity was increased. In contrast, in hypoxic rat hearts, CDS enzyme activity remained unaltered (3). The involvement of various underlying pathological mechanisms, the time period of hypoxia, in situ versus isolated hearts, and different animal species may account for such differential changes in enzyme activity. The committed step of CL biosynthesis is condensation of CDP-DG with sn-glycerol-3-phosphate to form PG phosphate, which is catalyzed by PGPS (15). PGPS activity was decreased in the RV and LV of PPHN animals. The reduction in activity was accompanied by reduced [1-14C]linoleate-labeled PG production in PPHN animals, indicating reduced PG synthesis. The final step of cardiac CL biosynthesis involves the conversion of CDP-DG and PG to CL, which is catalyzed by CLS (13). CLS activity was decreased in the LV and RV of PPHN animals. The lowered PGPS and CLS enzyme activities may also contribute to the decrease in CL in PPHN animals. Overall, the results indicate that decreased CDS, PGPS, and CLS activities in the LV and RV are directly linked with the reduced ability to synthesize CL during the development of cardiac hypertrophy in PPHN animals.

Fig. 5. Monolysocardiolipin (MLCL) acyltransferase (MLCL AT) activity, tafazzin (TAZ) mRNA expression, and Ca$^{2+}$-independent phospholipase A$_{2y}$ (iPLA$_{2y}$) mRNA expression in the LV and RV of newborn, control, and PPHN animals. A: mitochondrial fractions were prepared from newborn and 3-day-old control and PPHN piglets, and MLCL AT activity was determined. B and C: total RNA was prepared from newborn and 3-day-old control and PPHN piglets, and iPLA$_{2y}$ mRNA expression (B) and TAZ mRNA expression (C) were determined in the LV or RV. *P < 0.05 vs. newborn piglets; #P < 0.05 vs. control piglets.

Fig. 6. Tetralinoleoyl CL (L$_4$-CL) content, complex II + III activity, and citrate synthase activities in control and PPHN animals. Mitochondrial fractions were prepared from newborn and 3-day-old control and PPHN piglets, and L$_4$-CL content (A), complex II + III activity (B), and citrate synthase activity (C) were determined in the LV and RV. #P < 0.05 vs. control piglets.
In the heart, CL undergoes extensive remodeling to form linoleate-enriched CL (15). Previous studies (45, 53, 54) have demonstrated that linoleoyl enrichment of mitochondrial CL to L4-CL is achieved by TAZ and MLCL AT. A significant decrease in L4-CL was observed in the LV and RV of PPHN animals. The reduction in L4-CL was accompanied by a decrease in MLCL AT activity in the LV and a reduction in the mRNA expression of TAZ in the RV. Nonfunctional mutations in TAZ lead to decreased L4-CL content, destabilization of mitochondrial supercomplexes, mitochondrial dysfunction, and energy production and are responsible for the X-linked cardiосkeletal myopathy Barth syndrome (20, 32, 34). CL undergoes hydrolytic degradation via deacylation to MLCL via PLA2 (25). A reduction in iPLA2 activity may contribute to diminished cardiac function (31) and lower L4-CL during the development of HF in rats (60), and genetic ablation of iPLA2/H9253 in mice resulted in decreased L4-CL and abnormal mitochondrial function (35). We observed a decrease in iPLA2γ observed in the LV and RV of PPHN animals.

Fig. 7. Mitochondrial respiratory chain protein levels in newborn, control, and PPHN animals. Mitochondrial fractions were prepared from the RV and LV of newborn and 3-day-old control and PPHN piglets, and mitochondrial respiratory chain protein levels were determined by Western blot analysis as described in MATERIAL AND METHODS. A representative blot is shown. Lane 1, newborn LV; lane 2, control LV; lane 3, PPHN LV; lane 4, newborn RV; lane 5, control RV; lane 6, PPHN RV. The labeled bands are 53-kDa ATP synthase subunit α, 47-kDa complex III subunit core 2, 39-kDa complex IV subunit 1, 30-kDa complex II subunit 1p, and 20-kDa complex I subunit NDUFB8.

Fig. 8. Schematic showing alterations in CL biosynthesis and remodeling enzymes, levels of CL and L4-CL, and complex II + III activity, which contribute to RV hypertrophy and the resulting heart failure in PPHN. Small arrows indicate a reduction (↓) in CL enzymes or CL and L4-CL in the RV of PPHN piglets. PA, phosphatidic acid; PG, phosphatidylglycerol; PGP, phosphatidylglycerolphosphate.
bolic changes including reduced mitochondrial respiration (22). In erythropoietin-treated mice, an increase in cardiac complex II activity was associated with elevated state 3 respiration (2). In Barth syndrome lymphoblasts, a reduction in CL was associated with lowered complex II activity (54). In Jurkat cells, an elevation in CL mediated by stomatin-like protein 2 expression increased complex II activity and facilitated the assembly of respiratory chain components (5). Since impairment of mitochondrial energy-producing ability has previously been shown to be associated with the development of RV HF in monocrotaline-induced pulmonary hypertensive rats (6), it is conceivable that alterations of CL (which is essential to many mitochondrial energetic processes) could be a causative factor in the development of RV hypertrophy leading to RV HF in PPHN.

The normobaric hypoxic pig model of PPHN is useful in view of its reproducibility, similarity in histological findings to human PPHN, and applicability to clinical pathophysiology of perinatal hypoxic pulmonary vasospassm and sensitization (21, 52). If hypoxic exposure begins at birth, vascular function and histology remain consistent with PPHN for several days after a return to normoxia (12). On the basis of previous studies (48, 49, 52), it is possible that the changes in CL may be secondary to PPHN-induced overload. Hypoxic perfusion of rat hearts did not alter the CL pool size (3). In addition, total phospholipid content as well as individual fatty acid composition were unaltered under hypoxic conditions in hearts of coronary artery-ligated pigs (12). We observed decreases in iPLA2γ mRNA expression in the LV and RV of PPHN piglets. This is in agreement with a previous study (31) in which iPLA2 protein expression was decreased in the hearts of rats with congestive HF. Thus, the alterations in CL metabolism during the development of PPHN-mediated RV HF may be different than those observed in hypoxia-induced HF. A recent study (40) in intracoronary microembolization-induced HF dogs suggested that phosphorylation of specific complex IV subunits was responsible for the decreased mitochondrial oxidative phosphorylation in isolated cardiac mitochondrial fractions, but the content of CL and L4-CL remained unaltered. The differences in CL metabolism in the present study and the intracoronary microembolization model are likely related to differences in the choice of animal. In addition, infarctions produced by the intracoronary microembolization method were completely healed for 2–3 wk before CL analysis (42). However, we must keep in mind that L4-CL levels were reduced in LV explants from human HF patients, who develop HF over an extended period of time. This and other subsequent studies establish CL as a key player in the mitochondrial defect observed in the human form of this disease.

In summary, our data suggest that a reduction in L4-CL in the heart of PPHN piglets may contribute to reduced mitochondrial respiratory chain function in the RV, potentially leading to RV hypertrophy followed by right HF. The restoration of mitochondrial function as a potential therapeutic approach for the treatment of HF has been hypothesized (51), and further work is needed to determine if this could be accomplished by restoring the CL profile via modulation of CL biosynthetic and/or remodeling enzymes.

**REFERENCES**


H1424  REDUCED CARDIOLIPIN IN RIGHT HEART FAILURE