Cardiac dysfunction in mice lacking cytochrome-c oxidase subunit VIaH

NINA B. RADFORD,1 BANG WAN,2 ANGELA RICHMAN,2 LIDIA S. SZCZEPAJIAK,2 JIA-LING LI,2 KANG LI,2 KATHY PFIEFFER,3 HERMANN SCHÄGGER,3 DANIEL J. GARRY,2,4 AND RANDALL W. MOREADITH5
1The Cooper Clinic, Dallas 75230; 2Department of Internal Medicine, The University of Texas Southwestern Medical Center, Dallas, Texas 75390; 3Biochemie I, Zentrum der Biologischen Chemie, Universität-Klinikum, 60590 Frankfurt, Germany; 4Molecular Biology Department, The University of Texas Southwestern Medical Center, Dallas, Texas 75390–8573; 5ThromboGene, Chapel Hill, North Carolina 27514

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Radford, Nina B., Bang Wan, Angela Richman, Lidia S. Szczepaniak, Jia-Ling Li, Kang Li, Kathy Pfeiffer, Hermann Schägger, Daniel Garry, and Randall W. Moreadith. Cardiac dysfunction in mice lacking cytochrome-c oxidase subunit VIaH. Am J Physiol Heart Circ Physiol 282: H726–H733, 2002; 10.1152/ajpheart.00308.2001.—Cytochrome-c oxidase subunit VIaH (COX VIaH) has been implicated in the modulation of COX activity. A gene-targeting strategy was undertaken to generate mice that lacked COX VIaH to determine its role in regulation of oxidative energy production and mechanical performance in cardiac muscle. Total COX activity was decreased in hearts from mutant mice, which appears to be a consequence of altered assembly of the holoenzyme COX. However, total myocardial ATP was not significantly different in wild-type and mutant mice. Myocardial performance was examined using the isolated working heart preparation. As left atrial filling pressure increased, hearts from mutant mice were unable to generate equivalent stroke work compared with hearts from wild-type mice. Direct measurement of left ventricular end-diastolic volume using magnetic resonance imaging revealed that cardiac dysfunction was a consequence of impaired ventricular filling or diastolic dysfunction. These findings suggest that a genetic deficiency of COX VIaH has a measurable impact on myocardial diastolic performance despite the presence of normal cellular ATP levels.

transgenic animals; diastolic dysfunction; energy metabolism; nuclear magnetic resonance spectroscopy

CYTOCHROME-C OXIDASE (COX), the terminal enzyme complex of the mitochondrial electron transport chain, catalyzes the transfer of electrons from reduced cytochrome c to molecular oxygen and translocates protons across the mitochondrial inner membrane. The establishment of this electrochemical gradient across the membrane provides the driving force for ATP synthesis (7). Three-dimensional X-ray structural analysis reveals that the cytochrome-c oxidase enzymatic complex is composed of four subunits in Paracoccus denitrificans (16) and 13 subunits in the bovine heart (18, 40). Three of the thirteen subunits (I, II, and III) of mammalian cytochrome-c oxidase are encoded in mitochondrial DNA and constitute its catalytic core. The remaining 10 subunits (IV, Va, Vb, VIa, VIb, Vlc, VIIa, VIIb, VIIc, and VIII) are encoded in nuclear DNA and are less well understood (7), although many functional and structural features of the homologous subunits have been analyzed in the yeast system (1, 5, 9, 12, 21, 27, 35, 37, 39, 43).

Isoforms of subunits VIa, VIIa, and VIII, designated heart (H) and liver (L), exist in mammals that may function to modulate COX activity under various conditions of cellular metabolism (17, 19, 36, 44). The H form of subunit VIa (as well as VIIa and VIII) is expressed only in striated muscle, whereas the L form is more widely expressed (17, 36). The function of these isoforms cannot be studied in the yeast system, because only subunit Va and Vb isoforms, corresponding to subunit IV in mammals, exist (4). The location and protein interactions of subunit VIa at the interface of the two cytochrome-c oxidase monomers in the dimeric structure suggest that subunit VIa is involved in dimerization and possibly in modulation of the enzymatic properties. Currently, little is known about the enzymatic properties of COX isoforms of subunits VIIa and VIII, but evidence suggests that isoforms of subunit VIa, modulate COX activity and show different nucleotide dependence (2, 29). For example, COX activity in heart, but not in liver, was stimulated by ADP and this stimulation was abolished with preincubation of heart COX with an antibody against subunit VIaH (2). These results indicate that COX VIaH may be required for mediating the tissue-specific allosteric effects of ADP and ATP on the enzyme in striated muscle.

The transcript levels of nuclear genes encoding for VIaH and VIaL during embryogenesis and postnatal
METHODS

Generation of COXVIaH-deficient mice. The structure of the murine gene for VIaH has been previously described (41) (GenBank accession No. U34801) as has the developmental expression of the H and L subunits (26). Briefly, an expression cassette for neomycin phosphoglycerate kinase (PGK-neo, 1.7 kb) was inserted at the beginning of exon 2 at a SalI/EcoRV site, deleting 207 bp and truncating the 97 amino acid coding region of exon 2 at amino acid 28. A 1.8-kb herpes simplex virus-thymidine kinase cassette was inserted downstream of the 0.8-kb 3' flanking region of the gene, and the entire construct was linearized at a unique NotI site. The targeting vector consisted of 5.0-kb of 5' COXVIaH sequence, the neomycin cassette, a 0.8-kb fragment of 3' COXVIaH sequence, and a cassette encoding thymidine kinase drug resistance. Approximately 2.5 × 10⁷ J1 embryonic stem cells (kindly provided by R. Jaenisch, Whitehead Institute), after growth to near confluence were electroporated in a final volume of 0.75 ml with 50 μg of the linearized construct and plated on mitotically arrested mouse embryonic fibroblasts. Positive-negative selection was carried out as described (38). Neomycin-resistant and gancyclovir-sensitive clones were expanded, and DNA was isolated by standard techniques. Authentically targeted clones (targeting frequency 1:9) were identified by hybridization with the 300-bp probe isolated downstream of the 3' flank (Fig. 1), expanded, and injected into day 3.5 blastocysts from naturally mated C57BL/6 mice. After injection, the blastocysts were implanted into pseudo-pregnant Swiss outbred females (6–10 per female), and Agouti offspring were mated to wild-type C57BL/6 partners. Progeny from these matings were screened for the presence of the mutated allele (Fig. 1), and heterozygous animals were crossed to generate mice deficient in VIaH, as assessed by Southern analysis (Fig. 1A). Correctly targeted mice were identified by digesting genomic DNA (isolated from tail biopsies) with SacI (unique site introduced by insertion of the PGK-neo cassette in exon 2) to generate a wild-type allele.
fragment (5 kb) and a mutant allele fragment (4 kb) identified using the 300-bp probe and Southern blot analysis.

Northern analysis. Total RNA from mouse skeletal muscle and heart was prepared by directly homogenizing the tissues in 4 M guanidinium followed by CsCl density gradient centrifugation. RNA electrophoresis and filter hybridization were performed as described previously (24). Hybridization probes were synthesized by primer extension using sequence-specific primers and the entire COXVIaH cDNA template (22). Hybridization membranes were washed in 0.1× SSC/0.1% SDS at 60°C for 1 h (high stringency) to eliminate cross-reactivity between the H and L isoforms (data not shown).

Electrophoretic techniques, isolation of complex IV subunits, and protein sequencing. Blue native-PAGE (BN-PAGE), second-dimension Tricine-SDS-PAGE, staining, and densitometric quantification were undertaken as previously described (31). Heart muscle (10 μg wet weight) was homogenized and solubilized by the addition of 6 μl 10% dodecylmaltoside. After centrifugation at 100,000 g, the supernatant was applied to a 1-cm sample well for BN-PAGE (4–15% acrylamide gradient gel). The above protocol was scaled up 18-fold for sample preparation and protein sequence analysis. The visible blue bands after BN-PAGE, representing complex IV from wild-type and VIaH mutants, were excised from the gel and cut into six portions. These portions were loaded as a stack of six slices onto a Tricine-SDS gel (16% acrylamide containing 6 M urea). After electrophoresis, the proteins were blotted onto Immobilon P and sequenced directly using a 473A protein sequencer (Applied Biosystems) (30).

Sequence analysis. Sequence analysis was performed as previously described (31). The protein sequences were: WT VlαH: AASAAGDGDHHG; WT VIII-H: VSAKP; MUTANT VlαL: xxGAHGEEG (the two serines at the NH2-terminus could not be identified); and mutant VIII-H: VSAKP.

Biochemical assays. Hearts were isolated, quickly weighed, and frozen between aluminum tongs precooled in liquid nitrogen. Myocardial tissue was pulverized in a mortar cooled and frozen between aluminum tongs precooled in liquid nitrogen. Biochemical assays.

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Ultracut E microtome. Semithin (1 μm) sections were stained with toluidine blue and examined using light microscopy. Ultrathin sections (70 nm) were mounted on Formvar-coated copper oval slot grids, stained with uranyl acetate and lead citrate, and examined with a JEOL 1200EX II electron microscope at an accelerating voltage of 120 kV in the transmission mode.

Isolated working heart perfusion. Mice (25–30 g) were killed and the thoracic contents (washed immediately with 4°C perfusate) were excised and placed in 4°C perfusate. The heart and aorta were dissected free of extraneous tissue, and a fluid-filled cannula was threaded into the aorta and secured with a suture. Hearts were perfused in the Langendorff mode at 37.5°C with a retrograde perfusion pressure of 80 cmH2O (≈60 mmHg). Mouse hearts require a retrograde perfusion pressure higher than 50 mmHg to avoid slowly developing cardiac dysfunction (14). A cannula was inserted into a small opening in the left atrial (LA) wall and secured circumferentially with a suture. The preparation was then converted to a working mode with LA pressure (LAP) of 15 cmH2O and an anterograde perfusion pressure (APP) of 70 cmH2O (≈52 mmHg). An APP of 70 cmH2O was selected, because this was the highest APP tolerated by the COXVIaH mutant hearts and because the pressure has been successfully utilized in working heart preparations described previously (3). Aortic pressure was monitored through a sidearm connector attached to the aorta cannula for measures of arterial pressure and heart rate (and for gating as needed) with a multichannel polygraph (Colbourne Instruments; Lehigh Valley, PA) for cardiac perfusions utilizing nuclear magnetic resonance (NMR) spectroscopy. The nonrecirculating medium was a modified Krebs-Henseleit bicarbonate buffer (95% O2–5% CO2; pH 7.4) containing (in mM) 118 NaCl, 4.7 KCl, 2.5 NaHCO3, 1.2 MgSO4, 5.5 glucose, 2.5 CaCl2 and 0.5 Na2EDTA. Coronary and aortic flows were measured by timed collections (over 5-min intervals); cardiac output is the sum of coronary and aortic flows. These collections were obtained by diverting either aortic flow or coronary vein effluent into a graduated cylinder. Stroke work was computed as mean aortic pressure multiplied by stroke volume times 0.0136 (14). A tiny capillary tube with a known volume phosphate standard was positioned along the length of the heart within the NMR tube to be used as a standard for quantitation of metabolites from the spectral data. Left ventricular performance curves were generated using the isolated working heart preparation. At a constant APP of 70 cmH2O, the LAP was increased in a stepwise fashion from 15 to 20 to 25 cmH2O and stroke work response was measured. One set of experiments was performed on the bench, and a second set was performed inside the vertical bore of the 400-MHz spectrometer during NMR imaging.

NMR imaging. Hearts were perfused within the vertical narrow bore of a Bruker 9.4 T/8.9-cm (gradient-enhanced) spectroscopy system. High resolution, spin-echo images were obtained using a standard imaging sequence modified to accommodate a hard 180-degree pulse and cardiac triggering. Images of a 1-mm slice were collected in sagittal and transaxial planes during end systole and end diastole with a repetition time of 350 ms, echo time of 9 ms, 90° flip angle, number of acquisitions of 2, matrix size of 256 × 256 and a field of view of 17 mm. Volumes were calculated using the volume calculation for a prolate ellipse with variables of length in the sagittal plane and minor and major axes in the transaxial view using National Institutes of Health Image software (SymanTec).

31P NMR spectroscopy. Hearts were perfused in the working mode in the vertical narrow-bore of a 14.1 T/5.1-cm Oxford magnet interfaced with a high resolution Varian Inova spectrometer equipped with a 10-mm broadband probe that can be tuned to 23Na or 31P. The field homogeneity was...
optimized using the $^{23}$Na resonance until a line width of 10–15 Hz was obtained. Partially saturated $^{31}$P NMR spectra were averaged over 20 min using a 45-degree pulse of 15 μs, an interpulse delay of 665 ms, a 12-kHz spectral width, and 4-K time domain data points. Resonance areas obtained under these conditions were corrected for $T_1$ relaxation by factors determined from a fully relaxed spectrum. For NMR determination of ATP concentration, peak areas, corrected for saturation effects, were compared with a known reference standard for calculation of absolute concentration. After NMR data collection, total myocardial ATP was also measured using standard biochemical methods described above to verify the precision of the NMR methodology. NMR spectra were analyzed using NUTS software (Acorn NMR; Palo Alto, CA). Free-induction decays were baseline corrected, exponentially multiplied with 60 Hz of line broadening, Fourier transformed, and phase corrected manually. Peak areas were calculated using the automated line-fit procedure.

Statistical analyses. The spectrometer operator was blinded to the genotypes of all animals throughout the spectral and imaging data analysis until the data were grouped for pooled statistical analysis. Data are reported as means ± SD unless otherwise noted. Resonance areas for metabolites were compared using the repeated-measures analysis of variance with Tukey's test for comparison to control or with Dunnett's test for multiple comparisons among all groups. Hemodynamic data are compared using paired $t$ tests for multiple comparisons for 3 or more sequential measures. All statistical analyses are performed using an automated software program (TexasSoft; Cedar Hill, TX). $P < 0.05$ is considered significant.

RESULTS

Generation of mice with a null mutation in COX-VIaH. The murine COXVIaH gene was mutated by a replacement vector resulting in the deletion of two-thirds of exon 2 (Fig. 1A). Confirmation of a successfully targeted COXVIaH locus included an absence of full-length or truncated COXVIaH mRNA isolated from heart and skeletal muscle of the COXVIaH−/− mouse (Fig. 1C). The presence of β-actin mRNA, included as a control for RNA loading, demonstrated the integrity of the RNA in all the samples. Mice heterozygous or homozygous for the mutation demonstrated normal fecundity and a normal life span, and there was no evidence of embryonic loss of mutant mice (Table 1).

Lack of histochemical and ultrastructural changes in myocardial tissue from mutant mice. Sections of myocardial tissue stained with hematoxylin and eosin revealed no obvious structural differences between mutant and wild-type cardiac muscle. There were neither pericardial abnormalities nor increases in myocardial fibrosis in the mutant hearts (not shown). The ultrastructural evaluation of adult wild-type and mutant hearts revealed no qualitative differences in basic cellular ultrastructure including mitochondrial morphology, size, or number per cell (Fig. 2).

COX activity and subunit tissue content. The tissue contents of complexes I, III, and V (ATP synthase) in the knockout hearts were normal or slightly increased compared with wild-type levels (Fig. 3). However, the complex IV protein (COX) content (from densitometric quantification of two-dimensional gels) and COX activity was only 23% of wild-type levels. Direct protein sequence analysis of the subunit VIa-band in wild-type hearts revealed that this band was composed of two isoforms (about 80% VIaH and 20% VIaL, Fig. 4). However, the subunit VIa-band in the knockout mice was composed of exclusively the VIaL isoform. Because total complex IV from the knockout mouse was reduced to 23%, the tissue content of complex IV comprising subunit VIaL seems to be unchanged in knockout and wild-type mice (i.e., there is no apparent compensation by induction of the biosynthesis of subunit VIaL in the mutant hearts). Protein sequence analysis of subunit VIII revealed that the heart isoform VIII-H was retained in the knockout mouse and not replaced by VIII-L. A chimeric complex IV comprising subunits VIaL and VIII-H was composed of exclusively the VIaL isoform. Because total complex IV from the knockout mouse was normal or slightly increased compared with wild-type levels (Fig. 3), the complex IV comprising subunit VIaL seems to be unchanged in knockout and wild-type mice. COX enzymatic activity in the VIaH−/− heart was not significantly different compared with the wild-type heart.

Myocardial performance. Isolated hearts from both VIaH+/+ and VIaH−/− mice consistently generated stroke work when perfused with an APP of 70 and LAP of 15 cmH$_2$O. The hemodynamic variables from hearts perfused under these standard conditions are presented in Table 2. Left ventricular performance curves were generated using the isolated working heart preparation in hearts from VIaH+/+ and VIaH−/− mice (n = 7 for each group, Fig. 5). Hearts from VIaH−/− mice were unable to consistently generate stroke work in the isolated working heart under these conditions.
Initial perfusion conditions required for the stabilization of these hearts were an APP of 50 and a LAP of 25 cmH2O. Perfusion pressures were then adjusted slowly to an APP of 70 and a LAP of 15 cmH2O, which hearts simply could not tolerate (n = 20). To evaluate the contribution of systolic dysfunction to the failure of the working heart preparation in the hearts, null mice underwent echocardiography, which revealed the presence of normal left ventricular systolic function [fractional shortening of wild-type controls 0.57 ± 0.02 (n = 3); fractional shortening of COXVIaH mutants 0.56 ± 0.03 (n = 3)]. The failure of stroke work to increase with increasing LAP in the VIaH−/− hearts was a consequence of either impaired systolic function (inability of the heart to eject increasing ventricular filling volume) or impaired ventricular filling (inability of the heart to increase ventricular volume). To distinguish between these two possibilities, systolic function and ventricular filling (diastolic function) were assessed in isolated working heart preparation using magnetic resonance imaging in hearts from VIaH+/− and VIaH−/− mice. As a result of the high failure rate of the COXVIaH−/− working heart perfusion, this group was omitted from these technically challenging imaging studies.

![Graph](image)

**Fig. 3.** A: separation of native oxidative phosphorylation (OXPHOS) complexes from heart muscle by blue native-PAGE (BN-PAGE). Cardiac muscle (10 mg wet weight) from wild-type and COXVIaH knockout hearts were solubilized by deoxycholate and separated by a linear 4–16% acrylamide gradient gel for BN-PAGE, as described previously (31). The native complexes bind Coomassie dye and are visible during the electrophoresis. The OXPHOS complexes from solubilized bovine heart mitochondria were used as molecular mass standards (Std). The apparent mass of complex IV from mice is ~50 kDa larger than that of the bovine complex. Complex IV from the VIaH mutant mice is minimally detectable indicating a considerable specific reduction of the complex IV protein amount. B: quantification of OXPHOS protein amounts and COX activity in the subunit VIaH mutant hearts. The protein amounts of OXPHOS complexes in the subunit VIaH mutant hearts were determined by densitometric analysis of the Coomassie staining intensities of subunits of complexes in two-dimensional gels (Fig. 4) and compared with the staining intensities of the wild-type subunits. Complex I, III, and V protein amounts were increased by 36, 51, and 45%, respectively, whereas complex IV protein amount and COX activity were reduced to 23%.

![Graph](image)

**Fig. 4.** Two-dimensional resolution of the subunits of OXPHOS complexes. Lanes from the first dimension native gel (Fig. 3) were processed in a second dimension by Tricine-SDS-PAGE using 16% acrylamide gels containing 6 M urea as previously described (30). Comparison of the gels from (A) wild-type and (B) VIaH mutant hearts revealed a reduction of the complex IV protein amount in the mutant hearts and a concomitant loss of silver-staining of subunits I and III. *Protein sequencing of subunit VIa-bands revealed the presence of about 80% VIaH and 20% VIaL in the wild-type mice but exclusively VIaL in the mutant mice. Subunit VIII was retained as the heart form. The more intense silver-staining of subunit VIII in B, therefore, seems to be a silver-staining artifact. Other subunits of complex IV were assigned according to (31).

<table>
<thead>
<tr>
<th>Variable</th>
<th>VIaH+/+</th>
<th>VIaH−/−</th>
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<tbody>
<tr>
<td>Mean arterial pressure, mmHg</td>
<td>58 ± 8</td>
<td>49 ± 5</td>
</tr>
<tr>
<td>Cardiac output, ml/min</td>
<td>6.0 ± 0.8</td>
<td>6.9 ± 0.5</td>
</tr>
<tr>
<td>Stroke volume, µl</td>
<td>24 ± 3</td>
<td>26 ± 2</td>
</tr>
<tr>
<td>Heart rate, beats/min</td>
<td>251 ± 15</td>
<td>261 ± 12</td>
</tr>
<tr>
<td>LVED volume, µl</td>
<td>41 ± 7</td>
<td>46 ± 4</td>
</tr>
</tbody>
</table>

Values are means ± SD; n = 7.
atrial (LA) pressure was increased from 15 to 20 to 25 cmH2O. Stroke work did not significantly increase in hearts from mutant mice, whereas it increased significantly (*P < 0.05, 15 vs. 25 cmH2O) in the wild-type hearts.

Fig. 5. Left ventricular performance curves in the isolated working heart. Stroke work (means ± SD) was calculated in hearts from VlaH+/+ (denoted by ●) and VlaH +/- (denoted by ○) mice as left atrial (LA) pressure was increased from 15 to 20 to 25 cmH2O. Stroke work did not significantly increase in hearts from mutant mice, whereas it increased significantly (*P < 0.05, 15 vs. 25 cmH2O) in the wild-type hearts.

6, A and B, shows typical gated magnetic resonance images of an isolated working mouse heart in transaxial views at end diastole and end systole. Ejection fraction determined from left ventricular end-diastolic and end-systolic volumes under basal conditions was not significantly different in hearts from VlaH+/+ and VlaH+-/- mice (51 ± 1 vs. 51 ± 2%, respectively, n = 5) suggesting preserved systolic function in the COX-VlaH+/- heart. To assess ventricular filling, left ventricular end-diastolic volumes were measured at baseline and after an increase in LAP. Under basal conditions, there were no significant differences in mean arterial pressure, cardiac output, stroke volume, heart rate, or left ventricular end-diastolic volumes in VlaH+/+ and VlaH+/- hearts (Table 2). Figure 6C reveals the relationship between left ventricular end-diastolic volume and LAP in hearts from VlaH+/+ and VlaH+/- mice (n = 7 in each group). Whereas left ventricular end-diastolic volume increased significantly with increasing LAP in hearts from VlaH+/+ mice, there was no significant change in left ventricular end-diastolic volume in hearts from VlaH+/- mice, suggesting the presence of impaired ventricular filling. As in the previous studies, stroke work increased with increasing LAP in hearts from VlaH+/+ mice, whereas it remained constant in hearts from VlaH+/- mice.

Energy metabolite levels in myocardial tissue. The concentration of ATP was measured using both standard biochemical methods and 31P NMR spectroscopy with a reference standard. In Table 3, ATP concentrations are listed for wild-type, heterozygote, and homozygote mutant hearts. There are no differences in ATP concentration in the three groups measured by either method. Although ATP concentration is lower in work-performing hearts (measured by NMR and confirmed by standard biochemical methods), there were no differences among the three genotypes.

DISCUSSION

Effect of COXVlaH deficiency on cardiac function. To examine the functional significance of the cytochrome-c oxidase subunit VlaH on oxidative energy production and myocardial performance, we generated COXVlaH-deficient mice. The predominant cardiac phenotype resulting from gene disruption of COXVlaH is impaired left ventricular filling or diastolic dysfunction under maximal cardiac load. Abnormal left ventricular diastolic function in the absence of systolic dysfunction is the etiology of congestive heart failure in as many as one-third of patients who carry this diagnosis (8, 34). Although diastolic dysfunction is a common clinical diagnosis, simple animal models (i.e., diastolic dysfunction in the absence of dramatic systemic confounders such as hypertension, diabetes, or ischemia) that can be used to study metabolism and mechanical function are uncommon.

Table 3. Myocardial ATP concentration from VlaH+/+, VlaH+/-, and VlaH−/− mice

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Biochemical Method</th>
<th>n</th>
<th>31P MRS n</th>
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<tbody>
<tr>
<td>VlaH+/+</td>
<td>2.7 ± 0.3*</td>
<td>5</td>
<td>1.9 ± 0.4</td>
</tr>
<tr>
<td>VlaH+/-</td>
<td>3.0 ± 0.3</td>
<td>5</td>
<td>2.2 ± 0.3</td>
</tr>
<tr>
<td>VlaH−/−</td>
<td>3.1 ± 0.5</td>
<td>5</td>
<td>2.0 ± 0.6</td>
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Values are means ± SD measured biochemically in tissue and by 31P NMR spectroscopy in isolated working hearts (confirmed using biochemical methods on extracted tissue prepared from perfused hearts). *μmol/g per wet wt. MRS, magnetic resonance spectrometry.

Fig. 6. A: typical gated magnetic resonance images of the isolated working mouse heart in diastole (left) and systole (right). B: left ventricular end-diastolic volume (LVEDV, means ± SD) was determined from magnetic resonance images during diastole as LA pressure was increased from 15 to 20 to 25 cmH2O in hearts from VlaH+/+ (denoted by ●) and VlaH+/- (denoted by ○) mice. LVEDV did not significantly increase in hearts from mutant mice, whereas it increased significantly (*P < 0.05, 15 vs. 25 cmH2O) in the wild-type hearts.

Fig. 6. A: typical gated magnetic resonance images of the isolated working mouse heart in diastole (left) and systole (right). B: left ventricular end-diastolic volume (LVEDV, means ± SD) was determined from magnetic resonance images during diastole as LA pressure was increased from 15 to 20 to 25 cmH2O in hearts from VlaH+/+ (denoted by ●) and VlaH+/- (denoted by ○) mice. LVEDV did not significantly increase in hearts from mutant mice, whereas it increased significantly (*P < 0.05, 15 vs. 25 cmH2O) in the wild-type hearts.
Cellular mechanisms linking genotype to phenotype. A number of different etiologies for diastolic dysfunction have been described. Increased resistance to filling of ventricular chambers may be due to structural abnormalities such as constrictive pericarditis or myocardial fibrosis. Numerous cellular or metabolic causes of diastolic dysfunction have also been proposed. Impaired signaling or function of the sarcoplasmic reticulum ATP-dependent Ca\(^{2+}\) pump leading to abnormal intracellular Ca\(^{2+}\) homeostasis has been implicated in the setting of ischemia, hypertrophic cardiomyopathy, and chronic heart failure where systolic dysfunction is commonly present, as well (13, 25). Impaired restoration of diastolic Na\(^{+}\) levels has been implicated in postischemic hypertrophied hearts (23). Inhibition of glycolysis may cause diastolic dysfunction through a number of mechanisms: loss of glycolytically derived ATP, which is used preferentially for cardiac homeostasis (6, 28, 42); alterations in intracellular pH and P\(_i\) accumulation, which may influence the sensitivity of the myofilament to Ca\(^{2+}\) (15); and accumulation of sugar phosphates, which may have toxic effects on intracellular Ca\(^{2+}\) homeostasis (20). Given that nitric oxide has been shown to enhance left ventricular relaxation under normal and stressed conditions, from endogenous or exogenous sources respectively, inhibition of nitric oxide may also be linked to diastolic dysfunction via a cGMP-induced reduction in myofilament response to Ca\(^{2+}\) (10, 32).

In the present study, we observed that gene disruption of COXVIaH results in myocardial diastolic dysfunction and the characterization of this model addressed several of the possible causes. Using histological techniques, we have excluded structural causes of diastolic dysfunction, such as pericardial disease or intramyocardial fibrosis. The \(^{31}\)P NMR data suggest no significant differences in pH, sugar phosphates, or total myocardial ATP among VIaH/+, VIaH+/−, and VIaH−/− hearts. COX activity at rest is reduced only in hearts from VIaH−/− mice. We postulate that the enzymatic activity in the heterozygote may be reduced in the setting of increasing preload. Studies are currently underway to assess a number of other metabolic features in this model of diastolic dysfunction including Na\(^{+}\) and Ca\(^{2+}\) handling, nitric oxide activity, and oxidative versus glycolytic substrate utilization patterns.

Role of VIaH in COX function. Finally, what insight does this transgenic model provide regarding the role of subunit VIaH in modulating COX activity? Previous reports indicated that VIaH regulates COX activity via binding of ADP or ATP (2, 29). The present results using VIaH−/− mice indicate that the deletion of subunit VIaH also has dramatic effects on assembly/stability of COX so that COX activity is reduced considerably due to low COX protein amounts. Additional experiments are in progress to further define the effects of this mutation on the assembly and stability of COX enzyme. We previously observed that the turnover number of COX increased from 120 s\(^{-1}\) to 200 s\(^{-1}\) and the \(K_m\) for cytochrome c dropped from 38 \(\mu\)M to 25 \(\mu\)M when the liver isoforms (VIaL, VIIIIL) were replaced by the heart isoforms (VIaH, VIII-H) in the developing rat heart (31). Because VIa and VIII were exchanged on similar time scales, we could not discern whether subunit VIa or VIII, individually or collectively, were responsible for the altered enzymatic properties.

In summary, using gene disruption technology, we have produced mice that lack COX subunit VIaH. These mice are viable and display surprisingly subtle cardiac dysfunction in the form of impaired myocardial diastolic performance under conditions of maximal cardiac load. Further studies are in progress to define the cellular and molecular mechanisms underlying this distinctive phenotype, which may provide insight into therapeutic strategies in the treatment of patients with diastolic heart failure.

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